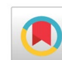

Evaluation on Antibacterial Activity and Hepatoprotectivity of *Rauwolfia serpentina*

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Abstract

Background: Rauwolfia serpentina plant known as Indian Snake Root is widely being utilized throughout the world as a traditional herbal plant capable of relieving various types of human infectious disease due to the presence of several types of bioactive compounds. Objective: The purpose of this study was to assess the antibacterial and hepatoprotective activity of 70% ethanolic leaf extract of Rauwolfia serpentina. The leaf extract of Rauwolfia serpentina improves the antibacterial activity and hepatoprotectivity by expressing its bioactive metabolites. Methods: The antibacterial activity was studied using disk diffusion method and hepatoprotective activity by liver function tests (LFT) and histological examination. The biochemical parameter of LFT includes Alanine aminotransferase (ALT) while haematoxylin and eosin staining was used to observe the morphology of the mice liver section. In this hepatoprotective study, about 20 mice were divided into four groups designated. All the groups were fed with plant extracts for 6 days. Results: In a mouse model of paracetamol-induced hapatotoxicity, ethanolic leaf extract of Rauwolfia serpentina showed a good response to hepatoprotective activity by rearrangement of the live-r architecture and decreasing the level of ALT. In addition, the ethanolic leaf extracts of Rauwolfia serpentina were tested against five different bacterial strains: Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aureginosa, and Proteus vulgaris. As a result, when compared to the conventional antibiotic, Gentamycin, the ethanolic extract of the plant demonstrated antibacterial activity. Conclusion: This study showed that 70% ethanolic leaf extract of Rauwolfia serpentina has antibacterial activity and hepatoprotective properties.

Key Words: Alanine aminotransferase, Antibacterial activity, Hepatoprotectivity, Paracetamol and Rauwolfia serpentina

Significance | Antibacterial activity and hepatoprotective effect of *Rauwolfia serpentina*

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Introduction

Metastatic Plants are an ancient source for bioactive compound usage such as pharmaceuticals, food additives, colours and flavours. Large number of the indigenous plant are being used as medical treatment to diseases and injuries because it consists of bioactive compound such as alkaloids, carbon compound, hydrogen, nitrogen, glycosides, gums, essential oils, tannins, fatty oils, mucilage, and resins. Most of the people are concerning on the medicinal plants as therapeutic agent. (Mallick et al., 2012).

Rauwolfia serpentina is commonly known as Sarpagandha, Indian snake root, and Harkaya. The plant belongs to Apocynaceae family and the origin is near to Indian Subcontinent and East Asia. Rauwolfia serpentina is an essential medicinal plant that widely

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plays role in both modern medical system and Ayurvedic medical system (Mallick et al., 2012). The alkaloids of *Rauwolfia serpentina* are categorizing into three groups which is reserpine, ajmaline and serpentine. The group of reserpine composes of rescinnamine, deserpine and other chemical compounds. Then, ajmalinine, ajmalicine and iso-ajmaline are undergo to ajmaline group. While, the group of serpentine is comprised of sepentinine, alstonine and many more. The roots, leaves and fruits of *Rauwolfia serpentina* obtain high percentage of secondary metabolites and also contributing in the Ayurvedic medical system for the treatment of various illness (Meena et al., 2009; Mittal et al., 2012; Poonam and Mishra, 2013). The root of *Rauwolfia serpentina* is used to treated for hypertension, insomnia, gastrointestinal disorders, mental agitation, epilepsy, anxiety, traumas, sedative, schizophrenia and insanity (Meena et al., 2009; Mittal et al., 2012). In addition, the roots juices or its extract can be provided treatment to liver disease, abdominal pain and remove the intestinal worm (Anisuzzaman et al., 2007; Nayak et al., 2004). While, the other disease such as malaria, pneumonia, burns, eczema, asthma, respiratory disease, skin cancers and spleen disease also treated with *Rauwolfia serpentina* plant (Anisuzzaman et al., 2007; Azmi et al., 2013; Bhattarai et al., 2009; Dey and De, 2011; Itoh et al., 2005; Mahanta et al., 2006; Rai, 2004).

Subsequently, the microorganism such as bacteria, fungus and viruses are involved as a main factor in causing the human serious infections around the world. In this century, the microbial resistance is being develop to the multiple of synthetic drug that commonly use as treatment to various disease (Gutmann et al., 1988; Tsuchiya et al.,1996). All of the resistance, affect the therapeutic activity on the patient and also sometimes develop other adverse effects on the host including hypersensitivity. Therefore, the researchers are concerning on the developing of alternatives antimicrobial agent for treatment of infections (Guillemot,1999). Medicinal plants are one of the best sources for natural antimicrobial agent, because it acts as higher efficient in treating the human infectious disease than synthetic antibiotics (Rashid et al., 2014). The bioactive components of plant are one of the compounds for reducing the development of antibiotic resistant human pathogens (Raghavendra et al., 2006). Many of the researchers reports the effectiveness of medicinal plants against on microorganisms and shows that plants are essential tool in generating of modern medicine (Evans et al., 2002). Most of the medical centres are widely utilized the medicinal plants because of its commercially availability and less cost. The antibacterial effectiveness of plant extract is associated with variety of components, such as aldehyde and phenolic compound (Lai and Roy, 2004).

Liver is an essential organ located in the vertebrates and other group of animals and it is the organ responsible for the detoxification of all the exogenous compounds such as xenobiotics, drugs, virus and alcohol. The liver is functioned in the biochemical pathways to fight with disease, supply nutrient, growth and bile reproduction (Ward and Daly, 1999). Basically, the liver is maintained the homeostasis of the human body and plays role in various of metabolic functions including metabolism of carbohydrates, lipid and proteins (Coppola et al., 2015). After the completion of intestinal absorption, blood is transported to the liver through the portal vein which is carried nutrients and different types of toxic substances such as drug, ethanol and other chemical compounds. This process may lead to the formation of severe toxicity and damage in the liver. Abnormality of the liver is differentiated as steatosis, fatty liver, liver cirrhosis and liver carcinoma (Coppola et al., 2015). Besides that, many synthetic drug and modern medicines which are used as treatment to liver disease, are less effective and cause various side effects (Rao et al., 2006). Therefore, the traditional medicinal plant become alternatives tool in hepatoprotective activity and effectivity of indigenous herbal plants are developed with pharmacological experimental studies (Shahani, 1999).

The present study was designed to investigate the antibacterial activity and hepatoprotective effect of 70% ethanol extract of leaf of *Rauwolfia serpentina* in paracetamol induced hepatic damage in mice.

Materials and Methods

Rauwolfia serpentina leaf crude extract preparation

Collection of *Rauwolfia serpentina*

The fresh leaves of *Rauwolfia serpentina* from *Apocynaceae* family were purchased from an authorized store. The leaves cleaned up by using a clean tap water. Then, cut it into small pieces and placed on the glass plate and allowed it to dry in completely. Once, the leaves dry completely, it was grinded into a course powder by using a proper industrial grinder and proceed to extraction process.

70% Ethanolic leaf extract of *Rauwolfia serpentina*

Soxhlet Extraction

The course powder of *Rauwolfia serpentina* leaves was performed in to Soxhlet extraction (Industrial laboratory equipment company, USA) with 70% of ethanol by using Soxhlet apparatus with heater at 40 ^{0}C. The course powder inserted about 12 g in each time along with some cotton wool to hold the powder and for ethanol absorbance. Totally of 16 cycles completed and each cycle took about 10 hours.

Rotary Evaporation

The extract obtained from Soxhlet extraction was performed rotary evaporation (CAT Scientific Laboratory, Germany) to remove any remaining of 70% ethanol. The extraction was placed about 300 mL into the round bottom flask and set at 62

revolutions per minute (rpm) and 47 ^{0}C temperature with some ice bags. Once the completion of rotary evaporation process, the plant extract formed into thick herbal paste.

Lyophilization

Prior to freeze drying process, the high concentrated extract was frozen by using laboratory freezer (Acson International, UK) at -20 ^{0}C for overnight. After that, the frozen extract was immediately performed lyophilization to avoid from melting by using freeze dryer (Labconco Corporation, USA) at temperature ranging at -48 ^{0}C to -52 ^{0}C and vacuum condition below than 0.133mBar at overnight. Finally, the 70% of ethanolic *Rauwolfia serpentina* leaf extract powder was stored in an airtight amber bottle with proper labelled and kept into desiccator to reduce surrounding humidity and for light protection (Azwanida, 2015). Advantage of freeze-drying is, it can produce high rate of phenolic compounds compare to air-drying process.

Antibacterial activity of leave extract of *Rauwolfia serpentina*

Microorganisms collection

The pure cultures of *Staphyloccus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae* were obtained from Microbiology Laboratory, Faculty of Medicine, Quest International University, Perak, Malaysia. All of these microorganisms were maintained under a Nutrient agar and stored in the refrigerator after a complete growth of these bacterial culture.

Inoculum Preparation

The nutrient broth was prepared by mixing with distilled water and stored in universal bottles under normal temperature. Then, 10 mL of broth was taken for each bacteria in the universal bottle by culturing a loop full of pure colony of bacteria into the bottle. The bacteria culture broth stored in the incubator under 37 ^{0}C of temperature for a day for bacterial growth.

Test Solution Preparation

The 70% ethanolic leaf extract of *Rauwolfia serpentina* plant powder was measured in different amount and mixed with 1 mL of ethanol (200 mg/mL, 300 mg/mL and 400 mg/mL). On that same moment, 1 g of the plant extract powder was diluted along with 1 mL of ethanol as a stock solution. The solution was placed into the sterilized Eppendorf tube and stored at 4 ^{0}C.

Muller Hinton Agar Preparation

The Muller Hinton Agar (MHA) was prepared about 500 mL by mixing distilled water. After that, it proceeded for sterilization at 121 ^{0}C by using autoclave machine. Next, the sterilized MHA poured into the sterile petri dish and allowed it to dry in completely followed by sealed the petri dish with paraffin tape to avoid any of contamination. Finally, it can proceed to utilize for further experiment.

Disc Diffusion Method

Prior to Disc diffusion method, the crude discs and Eppendorf tubes were sterilized by using autoclave. Then, the cultured bacteria swabbed on the sterilized MHA agar plate in uniform direction by using sterilized cotton swab. Following, placed the crude disc by immersing into the preparation of different concentration on plant extract on the agar plate in gently. Gentamycin antibiotic was used as positive control group while the crude disc with Dimethyl sulphoxide (DMSO) was categorized as negative control group in the disc diffusion test. Finally, placed the plate in the incubator at 37 ^{0}C for 24 hours to identify the zone of inhibition (ZOI) on all of these bacteria. The assay was performed with triplicates and the results were expressed as mean ± SD.

Cytotoxicity of leaves extract of *Rauwolfia serpentina*

Preparation of Dulbecco's Modified Eagle's Medium (DMEM)

The DMEM powder was added and mixed with 800 mL of double distilled water for measure out 1000 mL as a final volume concentration. Then, added 100 mL of fetal calf serum (FCS) by filtering with 0.4 μm of syringe filter into the solution. Subsequently, Amphotericin B (2 μg/mL) and Gentamycin (100 mg/mL) also were added and final volume was adjusted to 1000 mL with double distilled water. The procedure carried out in sterilized condition and complete growth medium stored in the refrigerator.

Cell culture

Human hepatocellular carcinoma (HepG2) cell line no. HB-8065 was obtained from the Eman Lab, University of Science, Malaysia. The cells were cultured with 10% DMEM complete growth medium and incubated at 37 ^{0}C with humid environment containing 5% of CO_2 under sterile condition.

Cell Revival

By following the technique of Freshney (2015), HepG2 cell line was revived and altogether about four vials of HepG2 cells were performed thawing process and transferred into the four different centrifuge tubes. Subsequently, 30 mL of 10% of DMEM with FCS was added into the each of centrifuge tube. After that, the cells were centrifuged by using a laboratory centrifuge (Thermo Fisher Scientific, USA) at 10,000 rpm about 10 minutes. Next, the supernatant of the media was discarded and the pellet resuspended with 10 mL of 1x Dulbecco's Phosphate Buffer Saline (DPBS) and centrifuged again at speed of 10,000 rpm for 10 minutes. The supernatant was discharged again and the present pellet was resuspended by using 10 mL of 1x DPBS. The last centrifugation process was performed for 5 minutes at 10,000 rpm by using laboratory centrifuge. Finally, the supernatant was discarded and the present cells were resuspended by using 10 mL of 10% of DMEM with FCS.

Cell Seeding

The process was performed by seeding about 2 mL resuspended of the HepG2 cells into the sterilized T-25 flasks. Then, about 3 mL of 10% of DMEM with FCS were mixed into each of the T-25 flasks by using a sterile Pasteur pipette and then placed into the CO_2 incubator (Heal Force 90, Shangai Lishen Scientific Equipment Corporation, China) and incubated at temperature of 37 ^{0}C and 5% of CO_2 atmosphere. The HepG2 cells were examined under the inverted phase of contrast microscope (Olympus Corporation, Japan) with the magnification of 40x for the complete formation of confluent monolayer of HepG2 cells.

Cytotoxicity Assay

HepG2 cell line was tested with the 70% of ethanolic extract of *Rauwolfia serpentina* and cytotoxicity was evaluated by using colorimetric 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay (Deepak et al., 2012).

3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay

The stock solution of *Rauwolfia serpentina* was prepared about (480 mg/mL) by mixing about 960 mg of 70% of ethanolic of *Rauwolfia serpentina* extract powder with about 2 mL of DMSO. Then, the solution was prepared into different level of concentrations (240, 120,60,30,15, 7.5, 3.75, 1.875, 0.9375 mg/mL) by following two-fold serial dilutions method. The 10% of DMEM complete growth media was categorized as blank while the HepG2 cells with complete growth media without adding plant extract was being as cell control.

As an aseptic method, about 50 μL of cultured HepG2 cells were took from T-25 flask and seeded into the 96-well plates at $2x10^4$ cells/mL of seeding density. 50 μL of different concentrations of the plant extract with the ranging from 0.9375 mg/mL to 240 mg/mL were transferred into the each of well and incubated for 24 hours, at 37 ^{0}C and 5% of CO_2. After that, 15 μL of MTT solution was added into the each well in the dark condition. The 96-well plate was covered up with aluminium foil and incubated for 4 hours at 37 ^{0}C with 5% of CO_2. Then added 100 μL of soluble solution into the each well and incubated for 1 hour, at 37 ^{0}C with 5% of CO_2 atmosphere. Finally, the 96-well plate was measured the absorbance at 570 nm by using the enzyme-linked immunosorbent assay (ELISA) microplate reader (CYBER Laboratory, USA).

The cytotoxicity of 70% of ethanolic extract of *Rauwolfia serpentina* was calculated from the following formula (Deepak et al., 2012).

Mean (Acc - As) × 100 = Percentage of cell growth inhibition (%) Mean (Acc)

Where Acc is mean absorbance of cell control and As is mean absorbance of test sample.

The cytotoxicity results were graphed and the half maximal of inhibitory concentration (IC50) was identified. The non-cytotoxic of 70% ethanolic *Rauwolfia serpentina* extract concentrations were proceeded to the hepatoprotective activity study. The assay was performed with triplicates and the results were expressed as mean ± SD.

Hepatoprotective activity *Rauwolfia serpentina* Leaves extract

Animal Model

BALB/c mice were studied on the hepatoprotective properties by using *Rauwolfia serpentina* leaves extract. The mice were kept in the Animal House at Faculty of Medicine, Quest International University, Perak, Malaysia. The mice were maintained in controlled temperature (25±1 ^{0}C) and under light-dark cycle (12:12 hours). The mice were got free access of drinking water and fed in a standard chow diet about 4 g for each. The studied was performed with Animal Protection agreement followed by the rules and regulation of Animal Ethical Committee, Government of Malaysia and signed by the ethical committee of Quest International University, Malaysia.

Paracetamol Dose Regimen

Each paracetamol tablet that contains about 500 mg were obtained from a reputed pharmacy. The dosage of administration to each mouse was set as 1 g/kg to develop hepatotoxicity. The paracetamol tablets were making into a fine powder formation by using mortar and pestle for dissolution in easily. Then, the paracetamol powder was suspended with the saline solution and administered in orally based on the body weight of each mice by using oral gavage.

Grouping of Mice and Treatments

In this research studies, about 20 mice were divided into four groups and therefore, each group was consisting about five mice. The first group of mice was undergone to normal control group which is receiving a single daily dose of 1 mL/kg of saline only via oral administration. The Group II categorized as negative control group which is administering a single daily dose of paracetamol (1 g/kg) only in orally. Subsequently, the Group III, classified as positive control group that is receiving a single daily dose of both 1 g/kg of paracetamol and 20 mg/kg of silymarin through oral administration in respectively. The Group IV was categorized as tested group which is administering in orally a single daily dose of both 1 g/kg of paracetamol and 20 mg/kg of 70% ethanolic *Rauwolfia serpentina* leaf extract in respectively. The silymarin and 70% of ethanolic *Rauwolfia serpentina* leaf extract was administered after three hours of paracetamol administration to the each of the mice. The experiment was being taken for 6 days and on the 7th day, all of the mice were sacrificed and dissected.

Liver Functions Test (LFT)

End of the experiment, the mice were sacrificed by following to the guidelines. The mice were given a sedative which is diethyl ether by soaking with cotton ball and placed into the desiccator. At beginning, all precaution was followed by wearing a proper personal protective equipment (PPE) such as laboratory coat, gloves and mask. Blood from each group of mice was collected by the cardiac puncture. After that, the blood was performed in centrifugation process at 2,000 rpm and at temperature of 4 ^{0}C for 10 minutes for the separation of serum. The serum was transferred into a sterilized Eppendorf tube and store at 4 ^{0}C of temperature to identify the ALT activity. The assay was performed with triplicates and the results were expressed as mean ± SD.

Liver biopsy of mice and stained using Hematoxylin and Eosin technique

The mice from each of group was dissected liver to observe the morphological changes on its liver. Once the liver harvested in carefully and placed the tissue into the 10% of buffered formalin respectively, for the fixation purpose at overnight. After that, processing of the liver tissues was done by using automatic tissue processor, ATP-700. The procedure of the tissue processing is shown in the table 1 respectively. Subsequently, the liver tissue was proceeded for embedding with paraffin wax at the temperature of 60 ^{0}C with moulds and placed it under -20 ^{0}C for overnight. Following on, the liver was performed sectioning process by using microtome with the thickness of 4 µm. Once a clear ribboning was obtained from each of the specimen, placed it on a water bath at 57 ^{0}C. An empty and clean microscopic glass slide were used to captured the tissue section and labelled in properly according to specific group. Then, allowed the slides to dry in completely for overnight at room temperature. According to the Haematoxylin and Eosin staining (H&E) protocol mention in the table 2, all of the specimen was stained and observed under the light microscope at 40x magnification.

Results

Extraction provide 70% of ethanolic *Rauwolfia serpentina* leaf extract

The *Rauwolfia serpentina* plant species employed in the various types of treatment of disease such as cardiovascular disease (Anitha and Kumari, 2006), hypertension (von-Poser et al.,1990), mental disorder (Noce et al.,1954), arrhythmia (Kirillova et al., 2001), psychiatric disease (Bhatara et al.,1997), high blood pressure (Vakil,1955) and breast cancer (Standford et al.,1986). For instance, methanolic leaf extract of *Rauwolfia serpentina* develop a good response of anti-diarrheal effects on castor oil-induced diarrhoea by decreasing the fluids volume and intestinal weight in the mice model (Ezeigbo et al., 2012). Furthermore, the ethanolic root and leaf extract of *Rauwolfia serpentina* was experimented by against on four types bacterial strains which is *Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and thus, the ethanolic extract of the plant shows effectiveness on antibacterial activity as compared to two standard of antibiotics which is Ampicillin and Streptomycin (Deshmukh et al., 2012).

The collection leaves of *Rauwolfia serpentina* plant were washed it with tap water and followed by allow it to dry in completely because it consists of bioactive enzymes which is produce its active compounds as well as the metabolic reaction (Das et al., 2010).

According to Handa et al., (2008), stated the principle of extraction is isolating the active constituents of a plant by using a specific solvent with a standard protocol. The method of Soxhlet extraction, was taken with small grinded sample (Figure 1) and placed it into the thimble that with a strength of filter paper which is can placed in the of the Soxhlet extraction apparatus. The solvent extraction heated up through the round bottom flask, developed the vaporization into the chamber that have sample and condensed into the condenser follow by it drip back, and also once the extraction liquid reach to the siphon arm and pass into the round bottom flask again (Azwanida, 2015) (Figure 2). In respective of Handa et al. (2008); Visht and Chaturvedi (2012) stated that there is filtration of the extract is absence and the fresh solvent extraction is carried through the transfer equilibrium with a solid form.

The percentage of extraction yield of 70% ethanolic leaf extract of *Rauwolfia serpentina* was identified as 5.33±0 (Table 3). According to Azwanida, (2015) the 70% of ethanol is commonly plays role in Soxhlet extraction for extracting purpose and to produce the plant extract. As an evidence, the total amount of methanolic root extract of *Rauwolfia serpentina* was at 5% g/g in a dried root powder formation (Azmi and Qureshi, 2012).

Antibacterial activity of zone of inhibition in *Rauwolfia serpentina* leaf extract against on microorganisms

In the present study, the 70% ethanolic leaf extract of *Rauwolfia serpentina* was tested to investigate its effectiveness on the antibacterial activity. The microorganisms that were being tested are *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus*. Previously, we were prepared the ethanolic *Rauwolfia serpentina* leaf extract in different concentration (50 and 100 µL/mL) through dilution technique for triplicate. But, there have absent of diameter of inhibition on the tested microorganisms. Research study done by Murthy and Narayanappa (2015) was assessed the antibacterial activity of methanolic and chloroform extracts of leaf and root of *Rauwolfia serpentina* by disc diffusion method. Here, antibacterial activity was detected against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacilus subtilis* and *Klebsiella*

Table 1: Tissue processing protocol

Station	Reagent	Duration
1	70% of ethanol	1.5 hour
2	80% of ethanol	1hour
3	90% of ethanol	1hour
4	Absolute alcohol	1hour
5	Absolute alcohol	1hour
6	Xylene	1hour
7	Xylene	2hour
8	Paraffin wax	1.5 hour
9	Paraffin wax	Overnight

Table 2: Hematoxylin and Eosin staining protocol

Station	Reagent	Duration
1	Xylene	2 minutes
2	Absolute ethanol	2 minutes
3	Absolute ethanol	2 minutes
4	Absolute ethanol	2 minutes
5	95% of ethanol	2 minutes
6	70% of ethanol	2 minutes
7	Running tap water	At least 2 minutes
8	Haematoxylin	3 minutes
9	Running tap water	At least 5 minutes
10	Eosin	2 minutes
11	95% of ethanol	20 times (dip the slides)
12	95% of ethanol	2 minutes
13	100% of ethanol	2 minutes
14	100% of ethanol	2 minutes
15	Xylene	2 minutes

Table 3: Extraction yield of 70% ethanolic leaf extract of *Rauwolfia serpentina*. All of the values are representing as a formation of mean ± standard deviation. Each test was conducted with triplicates.

Mass of feed in plant (g)	Mass of extract in plant (g)	Percentage of extraction yield (%)
12±0	0.64±0.35	5.33±0

Table 4: Zone of inhibition of *Rauwolfia serpentina* extract. PC: Positive control; NC: Negative control

Pathogen	Diameter of zone (mm) on gram positive and gram negative bacteria				
	200mg/mL	300mg/mL	400mg/mL	PC	NC
Escherichia coli	0	4	7	10	0
Klebsiella pneumoniae	0	3	6	10	0
Pseudomonas aeruginosa	2	5	7	10	0
Proteus mirabilis	0	3	6	10	0
Staphylococcus aureus	3	5	10	10	0

pneumoniae. It showed there was no zone of inhibition presents at 50 µL/mL concentration of leaf and root chloroform extracts on *Staphylococcus aureus* and *Bacilus subtilis*. However, the maximum zone of inhibition exhibits at 100 µL/mL concentration against all of the test organisms for both of leaf and root extracts. Thus, it was evaluated that all test bacteria are susceptible to methanolic extract than the chloroform extract. Therefore, in the current study, we prepared the ethanolic plant extract concentration by measuring into different amount and dissolved with 1 mL of ethanol solution (200 mg/mL,300 mg/mL and 400 mg/mL) to induce high concentration.

Based on the table 4 shows, *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* have no zone of inhibition at the concentration of 200 mg/mL but there has developed diameter of inhibition at the 300 mg/mL and 400 mg/mL of concentrations of the ethanolic plant extract. Among the three types of microbes that mentioned, *Escherichia coli* consists high potential of zone of inhibition (4 mm at 300 mg/mL and 7 mm at 400 mg/mL), while the *Klebsiella pneumonia* and *Proteus mirabilis* contain an equal rate of inhibition diameter (3 mm at 300 mg/mL and 6 mm at 400 mg/mL). Furthermore, *Pseudomonas aeruginosa* and *Staphylococcus aureus* consists lower zone of inhibition at 200 mg/mL of concentration as compared to other two different concentration of the ethanolic plant extract. Among these two types of bacteria, *Staphylococcus aureus* have better inhibition diameter than *Pseudomonas aeruginosa* in all of these concentrations of the ethanolic plant extract. However, *Staphylococcus aureus* obtained higher zone of inhibition than *Escherichia coli* in all of these concentrations and also the microbe able to achieve the diameter of inhibition almost equal to the Gentamycin which was utilized for positive control result. In contrast, *Staphylococcus aureus* has highest zone of inhibition in 70% ethanolic *Rauwolfia serpentina* leaf extract as compared to other tested microorganisms. Gram negative bacteria may consist high level of intrinsic resistant on many antimicrobial agents but present result indicated ethanolic root extract still able to against on gram negative bacterial strains (Deshmukh et al., 2012).

Based on the previous research studies, the ethanolic extracts of *Rauwolfia serpentina* have high potential on antibacterial activity. In respective of Negi et al. (2014) stated the antibacterial activity is effective with ethanolic root and leaf extract of *Rauwolfia serpentina* by opposing on *Escherichia coli, Salmonella typhi* and *Pseudomonas aureginosa*. According to Jigna et al. (2005), presented the aqueous extract of *Rauwolfia serpentina* express lesser antibacterial activity effective than the ethanolic plant extract. In addition, the ethanolic root extracts of *Rauwolfia serpentina* developed good antibacterial activity by opposing on *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis* and *Bacillus cereus* (Fazal et al., 2012; Jigna et al., 2005).

The cytotoxicity of *Rauwolfia serpentina* leaf extract on HepG2 cell line

In present study, the cell inhibitory assay was performed to identify the effects of cell inhibition of HepG2 cells (human liver cancer cell line) that were treated with different concentrations of ethanolic leaf extract of *Rauwolfia serpentina*. The HepG2 cells were experimented for 24 hours of duration and the cytotoxicity of the experiment was calculated by against to untreated HepG2 cells. This experiment was conducted by using MTT assay to determine the cytotoxicity level of ethanolic leaf extract of *Rauwolfia serpentina*. Based on the Tetrazolium dye reduction, the metabolic activity of HepG2 cells can be determined. ELISA reader plays role in MTT assay to read the absorbance of 96- microwell plate at 570 nm and to evaluate the cell toxicity of the ethanolic plant extract.

The principle of MTT assay is known as a colorimetric assay which is identifying the metabolic reaction of cells and nicotinamide adenine dinucleotide phosphate (NADPH) cofactor-dependent cellular mitochondrial oxidoreductase enzyme will determined the condition of the viable cells (Stockert et al., 2018). In other words, according to Berridge (2005), the enzyme has potential to reduce the intensity of tetrazolium dye into the insolubilize of formazan where, it is in purple colour of appearance. As an evidence, determination of cytotoxicity for anticancer activity was performed by using MTT assay along with some cancerous cell line, including HepG2 cells, MCF7 cells and VERO cells and it indicated, evaluation of cell proliferation can be find based on viable cells metabolism (Senthilraja and Kathiresan, 2015). The MTT assay and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium) assays are largely utilized to analyse the proliferation of cell, involving determination the effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and identify new anti-cancer drug (Hayon et al., 2003).

Importantly, the cytotoxicity result is displaying as IC50 formation that is the half maximal of inhibitory concentration which is inhibited about 50% of the growing of HepG2 cells. The ethanolic leaf extract of *Rauwolfia serpentina* showed the IC50 value of 20 mg/mL, where it is possible to kill the HepG2 cells on this concentration (Figure 3). The American National Cancer Institute guidelines (NCI) was fixed the limitation for cytotoxicity of crude extracts at 50% inhibition of cell proliferation (IC50 value) about lower than 30 mg/mL after tested for 24 hours (Razak et al., 2007). By following, the concentration of 20 mg/mL that had stated in IC50, was administer to the mice by calculating its body weight in the tested control group via oral administration using clean oral gavage.

The cytotoxicity of *Rauwolfia serpentina* leaf extract on HepG2 cell line

Figure 1. Grinded powder of *Rauwolfia serpentina* leaves. This powder was proceeded to Soxhlet extraction process.

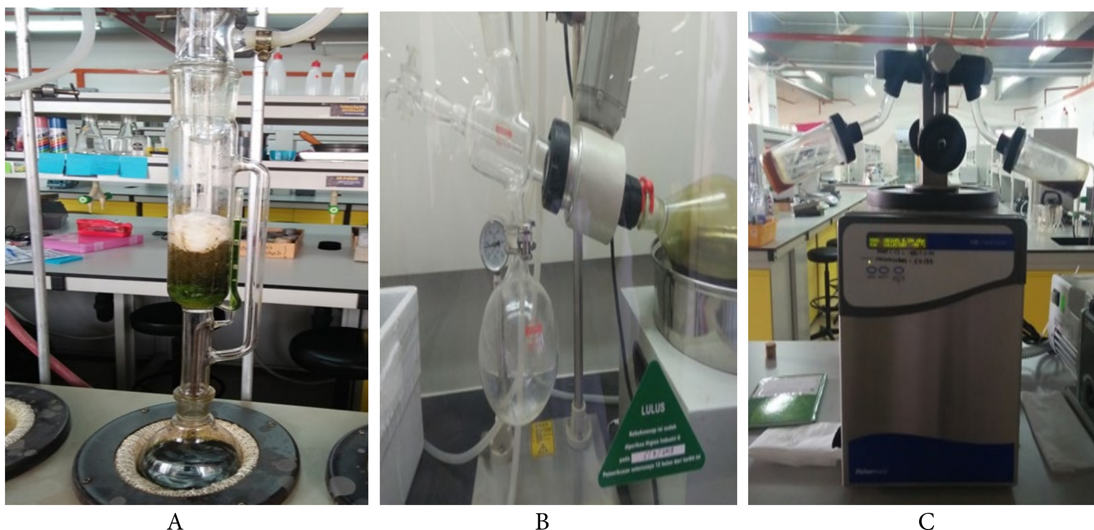

Figure 2. 70% of ethanolic leaf extraction process of Rauwolfia serpentina. Soxhlet extraction, (A); Rotary Evaporation, (B); Freeze Drying (lyophilization), (C).

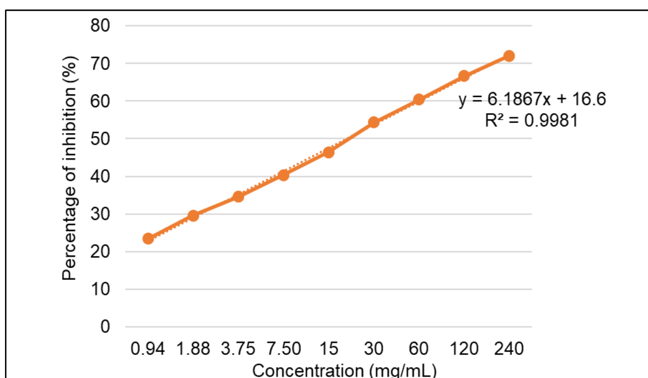

Figure 3. Cytotoxicity of Rauwolfia serpentina extract. MTT assay of HepG2 cell line treated with different concentration of plant extract. IC50: Half maximal of inhibitory concentration.

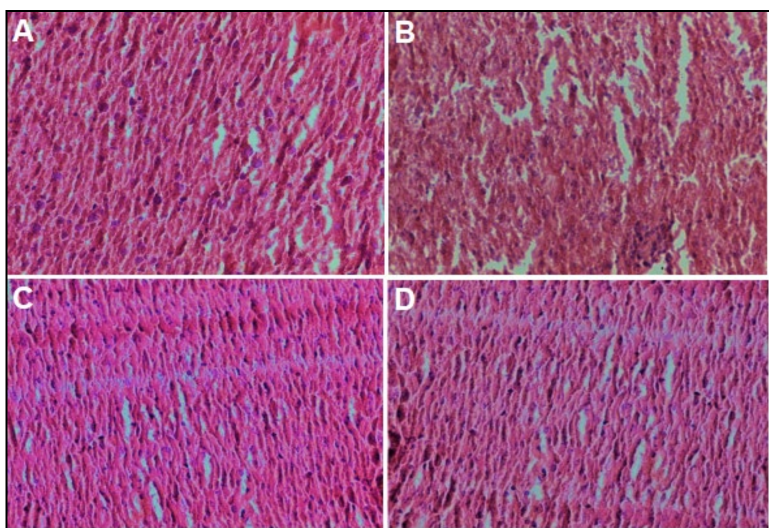

Figure 4. Effect of Rauwolfia serpentina leaf extract on the histological evaluation of liver biopsy. Photomicrographs sections of mice liver from each group. The H&E was used to staining the liver tissues. Normal control, (A); Negative control (B); Positive control, (C); Test control, (D). All of these pictures are representing the five mice model in each group under 40x magnification.

In present study, the cell inhibitory assay was performed to identify the effects of cell inhibition of HepG2 cells (human liver cancer cell line) that were treated with different concentrations of ethanolic leaf extract of *Rauwolfia serpentina*. The HepG2 cells were experimented for 24 hours of duration and the cytotoxicity of the experiment was calculated by against to untreated HepG2 cells. This experiment was conducted by using MTT assay to determine the cytotoxicity level of ethanolic leaf extract of *Rauwolfia serpentina*. Based on the Tetrazolium dye reduction, the metabolic activity of HepG2 cells can be determined. ELISA reader plays role in MTT assay to read the absorbance of 96- microwell plate at 570 nm and to evaluate the cell toxicity of the ethanolic plant extract.

The principle of MTT assay is known as a colorimetric assay which is identifying the metabolic reaction of cells and nicotinamide adenine dinucleotide phosphate (NADPH) cofactor-dependent cellular mitochondrial oxidoreductase enzyme will determined the condition of the viable cells (Stockert et al., 2018). In other words, according to Berridge (2005), the enzyme has potential to reduce the intensity of tetrazolium dye into the insolubilize of formazan where, it is in purple colour of appearance. As an evidence, determination of cytotoxicity for anticancer activity was performed by using MTT assay along with some cancerous cell line, including HepG2 cells, MCF7 cells and VERO cells and it indicated, evaluation of cell proliferation can be find based on viable cells metabolism (Senthilraja and Kathiresan, 2015). The MTT assay and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium) assays are largely utilized to analyse the proliferation of cell, involving determination the effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and identify new anti-cancer drug (Hayon et al., 2003).

Importantly, the cytotoxicity result is displaying as IC50 formation that is the half maximal of inhibitory concentration which is inhibited about 50% of the growing of HepG2 cells. The ethanolic leaf extract of *Rauwolfia serpentina* showed the IC50 value of 20 mg/mL, where it is possible to kill the HepG2 cells on this concentration (Figure 3). The American National Cancer Institute guidelines (NCI) was fixed the limitation for cytotoxicity of crude extracts at 50% inhibition of cell proliferation (IC50 value) about lower than 30 mg/mL after tested for 24 hours (Razak et al., 2007). By following, the concentration of 20 mg/mL that had stated in IC50, was administer to the mice by calculating its body weight in the tested control group via oral administration using clean oral gavage.

Morphological examination of liver in mice model

Histopathological examination of liver sections of mice from each group indicated in figure 4. The typical morphology of the liver tissue was observed in the normal control group (Group I) with location of nucleus and arrangement of hepatocytes were in normal state and no centrilobular changes occurred. The negative control group (Group II) showed early stages of nucleus changes by clumping into a position and centrilobular changes followed by the arrangement of hepatocytes in abnormal condition. In the positive control group (Group III) arrangement of hepatocytes developed in a good state and absence of nucleus clumping followed by presence of very minor centrilobular changes were observed. Besides that, the histological examination of the tested control group (Group IV) was displayed the improvement of hepatocytes arrangement and the appearance in normal condition of nucleus with small lobular changes. All of these tested substances which saline, paracetamol, silymarin and ethanolic *Rauwolfia serpentina* leaf extract were administer based on the mice body weight scale from each group.

Recknagel et al. (1989), mentioned that paracetamol is one of the susceptible drugs which induced liver injuries and also commonly utilized for detection of hepatoprotective activity. According to study done by Shahid and Subhan (2014), reported that acetaminophen (paracetamol) drugs were involved and developed histopathological changes in the liver of mice, including hepatocytes necrosis, extensive haemorrhage, nuclear changes, hydropic degeneration, congestion in the central vein and microvascular steatosis.

Furthermore, based on evidence found in histological examination, the administration of biherbal ethanolic extract (BHEE) has potential to develop hepatoprotection from the paracetamol induced acute liver injury in rats (Anantha et al., 2012). In the present study, silymarin was used as positive control to detect the hepatoprotectivity in paracetamol induced hepatotoxicity. Silymarin is one of the polyphenolic flavonoids that isolated from fruit and seeds of *Silybum marianum* (Valenzuela and Garrido, 1994) Silymarin exhibits antioxidant activity and also effective in hepatoprotection by obstructing the CCl_4 induced lipid peroxidation (Facino et al., 1990; Letteron et al., 1990).

Biochemical parameters in the mice liver

In the present study, serum level of Alanine aminotransferase (ALT) increased in the negative control group of BALB/c mice. While, the positive control group which is the mice treated with silymarin, decreased the ALT level compare to Group II. Besides that, the test control group also decreased the level of ALT and it occurred within the normal range (Figure 5). Thus, the 70% ethanolic leaf extract of *Rauwolfia serpentina* is a very effective source to prevent the paracetamol induced toxic effects in the mice liver.

The liver function test was demonstrated commonly by detection of bilirubin, albumin, prothrombin and many more parameters but aspartate aminotransferase is playing role as biomarker to identify the liver injury (Johnston, 1999). Whenever, there is

declined of biochemical content is occurred, it may because of increase the development of glucuronidation or inhibit the cytochrome P450 in the mice liver (Huber et al., 2008).

For instance, plant extract is used as therapeutic application to prevent the free radical pathogenesis in the liver of mice model (Bandyopadhyay et al., 1999). Restore the biochemical parameter within the normal range is indicated the hepatoprotective activity of the ethanolic plant extract against the paracetamol induced hepatotoxicity in rats (Oyagbemi and Odetola, 2010).

Based on the previous study, the methanolic root extract of *Rauwolfia serpentina* has potential to develop the antioxidant activity by increasing the levels of glutathione peroxide, glutathione reductase, catalase, glutathione-S-transferase and superoxide dismutase and declining the lipid peroxidation against the CCL_4 induced liver toxicity in albino rat models (Gupta et al., 2015).

Conclusion

This research study is offered the evidence that *Rauwolfia serpentina* plant species is effective on antibacterial activity and hepatoprotective activity. In the experiment with antibacterial activity, *Staphylococcus aureus* showed highest zone of inhibition among other types of microbes. Furthermore, when the concentration of 70% ethanolic leaf extract of *Rauwolfia serpentina* was increased, the level of antibacterial activity also increased. Histopathological examination exhibited administration of plant extract, restore the normal architecture of the liver in mice. Biochemical study showed treated with plant extract decreased the level of ALT to normal value. Isolation and identification of major and minor phytochemcial constituents of the *Rauwolfia serpentina* and possible mechanism for hepatoprotectivity activity of paracetamol induced liver toxicity by *Rauwolfia serpentina* are recommended for the future studies.

Abbreviations

LFT Liver function tests
ALT Alanine aminotransferase
rpm Revolutions per minute
mBar millibar
mL millilitre
^{0}C Centigrade
mg/mL milligram per millilitre
g Gram
mg milligram
MHA Muller Hinton agar
DMSO Dimethyl sulphoxide
ZOI Zone of inhibition
DMEM Dulbecco's Modified Eagle's medium
FCS Fetal calf serum
µm micrometre
µg/mL microgram per millilitre
HepG2 Human liver cancer cell line
CO_2 Carbon dioxide
DPBS Dulbecco's phosphate buffer saline
MTT 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide
µL microlitre
nm nanometre
ELISA Enzyme immunosorbent Assay
Acc Absorbance of cell control
As Absorbance of test samples
IC50 Half maximal of inhibitory concentration
SD Standard Deviation
BALB/c Albino, laboratory-bred strain of the house mouse
g/kg gram per kilogram
mL/kg millilitre per kilogram
mg/kg milligram per kilogram
PPE Personal protective equipment
H&E Haematoxylin and Eosin
g/g gram per gram
µL/mL microlitre per millilitre
mm millimetre
PC Positive control
NC Negative control
NADPH Nicotinamide adenine dinucleotide phosphate
MCF7 Human breast cancer cell line
VERO Lineage of cells
MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- Tetrazolium
ROS Reactive oxygen species
RNS Reactive nitrogen species
NCI American National Cancer Institute
BHEE Biherbal ethanolic extract
CCL_4 Carbon tetrachloride
U/L Units per Litre

Author Contributions

GH,YR, MK, have designed the experiments. GH,YR,FSRA, MK,PB and SR, have conducted the experiments, collected and analyzed the data. GH,YR,FSRA, MK and PB have drafted the manuscript. All the authors have read and approved the final manuscript.

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Competing financial interests

Authors declare no competing interest.

Supplementary Information

None

References

Anantha, K. C. D., Siva, R. C., & Manohar, R. A. (2012). Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in albino rats. Journal of Ayurveda and Integrative medicine, 3(4), 198-203.

Anisuzzaman, M., Rahman, A. H. M. M., Harun-Or-Rashid, M., Naderuzzaman, A. T. M., & Islam, A. K. M. R. (2007). An ethnobotanical study of Madhupur, Tangail. Journal of Applied Sciences Research, 3(7), 519-530.

Anitha, S., & Kumari, B. R. (2006). In vitro flowering in Rauvolfia tetraphylla L. Pakistan Journal of Biological Sciences, 9(3), 422-424..

Azmi, M. B., & Qureshi, S. A. (2012). Methanolic root extract of Rauwolfia serpentina benth improves the glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice. Advances in pharmacological sciences, 2012, 1-11.

Azmi, M. B., & Qureshi, S. A. (2013). Rauwolfia serpentina ameliorates hyperglycemic, haematinic and antioxidant status in alloxan-induced diabetic mice. Journal of Applied Pharmaceutical Science, 3(7), 136-141.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants, 4(3), 1-6.

Bandyopadhyay, U., Das, D., & Banerjee, R. K. (1999). Reactive oxygen species: oxidative damage and pathogenesis. Current Science-Bangalore, 77, 658-666.

Berridge, M. V., Herst, P. M., & Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnology annual review, 11, 127-152.

Bhatara, V. S., Sharma, J. N., Gupta, S., & Gupta, Y. K. (1997). Images in psychiatry. Rauwolfia serpentina: the first herbal antipsychotic. The American journal of psychiatry, 154(7), 894.

Bhattarai, S., Chaudhary, R. P., & Taylor, R. S. (2009). Ethno-medicinal plants used by the people of Nawalparasi District, Central Nepal. Our Nature, 7(1), 82-99.

Coppola, N., Pisaturo, M., Sagnelli, C., Onorato, L., & Sagnelli, E. (2015). Role of genetic polymorphisms in hepatitis C virus chronic infection.World journal of clinical cases, 3(8), 727-742.

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of medicinal plants research, 4(2), 104-111.

Deepak, H. B., Chandrasekaran, C. V., Dethe, S., Mundkinajeddu, D., Pandre, M., Balachandran, J., & Agarwal, A. (2012). Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacognosy Magazine, 8, 116-123.

Deshmukh, S. R., Ashrit, D. S., & Patil, B. A. (2012). Extraction and evaluation of indole alkaloids from Rauwolfia serpentina for their antimicrobial and antiproliferative activities. Int J Pharm Pharm Sci, 4(5), 329-334.

Dey, A., & De, J. N. (2011). Ethnobotanical aspects of Rauvolfia serpentina (L). Benth. ex Kurz. in India, Nepal and Bangladesh. Journal of Medicinal Plants Research, 5(2), 144-150.

Evans, C. E., Banso, A., & Samuel, O. A. (2002). Efficacy of some nupe medicinal plants against Salmonella typhi: an in vitro study. Journal of Ethnopharmacology, 80(1), 21-24.

Ezeigbo, I. I., Ezeja, M. I., Madubuike, K. G., Ifenkwe, D. C., Ukweni, I. A., Udeh, N. E., & Akomas, S. C. (2012). Antidiarrhoeal activity of leaf methanolic extract of Rauwolfia serpentina. Asian Pacific journal of tropical biomedicine, 2(6), 430-432.

Facino, R. M., Carini, M., Franzoi, L., Pirola, O., & Bosisio, E. (1990). Phytochemical characterization and radical scavenger activity of flavonoids from Helichrysum italicum G. Don (Compositae). Pharmacological research, 22(6), 709-721.

Fazal, H., Ahmad, N., Abbasi, B. H., & Abbass, N. (2012). Selected medicinal plants used in herbal industries; their toxicity against pathogenic microoraganisms. Pak. J. Bot, 44(3), 1103-1109.

Freshney, R. I. (2015). Culture of animal cells: a manual of basic technique and specialized applications, sixth ed. Wiley-Blackwell, New Jersey.

Guillemot, D. (1999). Antibiotic use in humans and bacterial resistance. Current opinion in microbiology, 2(5), 494-498.

Gupta, A. K., Irchhaiya, R., Chitme, H. R., & Misra, N. (2015). Free radical scavenging activity of Rauwolfia serpentina rhizome against CCl4 Induced liver injury. International Journal of Pharmacognosy, 2(3), 123-126.

Gutmann, L., Billot-Klein, D., Williamson, R., Goldstein, F. W., Mounier, J., Acar, J. F., & Collatz, E. (1988). Mutation of Salmonella paratyphi A conferring cross-resistance to several groups of antibiotics by decreased permeability and loss of invasiveness. Antimicrobial agents and chemotherapy, 32(2), 195-201.

Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction Technologies for Medicinal and Aromatic Plants,(First ed.), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.

Hayon, T., Dvilansky, A., Shpilberg, O., & Nathan, I. (2003). Appraisal of the MTT-based assay as a useful tool for predicting drug chemosensitivity in leukemia. Leukemia & lymphoma, 44(11), 1957-1962.

Huber, W. W., Rossmanith, W., Grusch, M., Haslinger, E., Prustomersky, S., Peter-Vörösmarty, B., ... & Schulte-Hermann, R. (2008). Effects of coffee and its chemopreventive components kahweol and cafestol on cytochrome P450 and sulfotransferase in rat liver. Food and chemical toxicology, 46(4), 1230-1238.

Itoh, A., Kumashiro, T., Yamaguchi, M., Nagakura, N., Mizushina, Y., Nishi, T., & Tanahashi, T. (2005). Indole alkaloids and other constituents of Rauwolfia serpentina. Journal of natural products, 68(6), 848-852.

Jigna, P., Rathish, N., & Sumitra, C. (2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. Indian Journal of Pharmacology, 37(6), 408-409.

Johnston, D. E. (1999). Special considerations in interpreting liver function tests. American family physician, 59(8), 2223-2230.

Kirillova, N. V., Smirnova, M. G., & Komov, V. P. (2001). Sequential isolation of superoxide dismutase and ajmaline from a tissue culture of Rauwolfia serpentina Benth. Prikladnaia biokhimiia mikrobiologiia, 37(2), 181-185.

Lai, P. K., & Roy, J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. Current medicinal chemistry, 11(11), 1451-1460.

Lettéron, P., Labbe, G., Degott, C., Berson, A., Fromenty, B., Delaforge, M., ... & Pessayre, D. (1990). Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice: evidence that silymarin acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochemical pharmacology, 39(12), 2027-2034.

Mahanta, R. K., Rout, S. D., & Sahu, H. K. (2006). Ethnomedicinal plant resources of Similipal biosphere reserve, Orissa, India. Zoos Print J, 21(8), 2372-2374.

Mallick, S. R., Jena, R. C., & Samal, K. C. (2012). Rapid in vitro multiplication of an endangered medicinal plant sarpgandha (Rauwolfia serpentina). American Journal of Plant Sciences, 3(4), 437-442.

Meena, A. K., Bansal, P., & Kumar, S. (2009). Plants-herbal wealth as a potential source of ayurvedic drugs. Asian Journal of Traditional Medicines, 4(4), 152-170.

Mittal, D. B., Meenakshi, D., Sharma, D. A., & Gothecha, V. K. (2012). Phytochemical & pharmacological acitivity of Rauwolfia serpentina–A review. International Journal of Ayurvedic and Herbal Medicine, 2(3), 427-434.

Murthy, K. M.S., & Narayanappa, M. (2015). Invitro study of antibacterial activity of leaf and root extract of Rauvolfia serpentina against gram positive and negative bacterial strains. International Journal of Recent Research in Interdisciplinary Sciences, 2(3), 33-37.

Nayak, S., Behera, S. K., & Misra, M. K. (2004). Ethno-medico-botanical survey of Kalahandi district of Orissa. Indian Journal of Traditional Knowledge, 3(1), 72-79.

Negi, J. S., Bisht, V. K., Bhandari, A. K., Bisht, D. S., Singh, P., & Singh, N. (2014). Quantification of reserpine content and antibacterial activity of Rauvolfia serpentina (L.) Benth. ex Kurz. African Journal of Microbiology Research, 8(2), 162-166.

Noce, R. H., Williams, D. B., & Rapaport, W. (1954). Reserpine (Serpasil) in the management of the mentally Ill and mentally retarded: Preliminary report. Journal of the American Medical Association, 156(9), 821-824.

Oyagbemi, A. A., & Odetola, A. A. (2010). Hepatoprotective effects of ethanolic extract of Cnidoscolus aconitifolius on paracetamol-induced hepatic damage in rats. Pakistan Journal of Biological Sciences, 13, 164-169.

Poonam, A. S., & Mishra, S. (2013). Physiological, biochemical and modern biotechnological approach to improvement of Rauwolfia serpentina. Journal of Pharmacy and Biological Science, 6(2), 73-78.

Raghavendra, M. P., Satish, S., & Raveesha, K. A. (2006). Phytochemical analysis and antibacterial activity of Oxalis corniculata; a known medicinal plant. My Sci, 1(1), 72-78.

Rai, S. K. (2004). Medicinal plants used by Meche people of Jhapa district, eastern Nepal. Our Nature, 2(1), 27-32.

Rao, G. M. M., Rao, C. V., Pushpangadan, P., & Shirwaikar, A. (2006). Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of ethnopharmacology, 103(3), 484-490.

Rashid, M. M. O., Amin, M. R., Mehadi, M., Chowdhury, H., Rahman, M. A., & Shamim, M. (2014). Comparative study on the antibacterial activity of four medicinal plants leaves of different ages. Int J Pharmacol Sci Invent, 3, 26-32.

Razak, M. F. A., Aidoo, K. E., & Candlish, A. G. (2007). Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. Tropical biomedicine, 24(2), 49-59.

Recknagel, R. O., Glende Jr, E. A., Dolak, J. A., & Waller, R. L. (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacology & therapeutics, 43(1), 139-154.

Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. J Appl Pharm Sci, 5(3), 080-084.

Shahani, S. (1999). Evaluation of hepato-protectivity efficacy of APLC: A herbal formulation in vivo in rats. Indian drugs, 36(10), 628-631.

Shahid, M., & Subhan, F. (2014). Comparative histopathology of acetaminophen induced hepatotoxicity in animal models of mice and rats. Pharmacology Online, 3(33), 32-43.

Stanford, J. L., Martin, E. J., Brinton, L. A., & Hoover, R. N. (1986). Rauwolfia use and breast cancer: a case-control study. Journal of the National Cancer Institute, 76(5), 817-822.

Stockert, J. C., Horobin, R. W., Colombo, L. L., & Blazquez-Castro, A. (2018). Tetrazolium salts and formazan products in cell biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta histochemica, 120(3), 159-167.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., ... & Iinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. Journal of ethnopharmacology, 50(1), 27-34.

Vakil, R. J. (1955). Rauwolfia serpentina in the treatment of high blood pressure: a review of the literature. Circulation, 12(2), 220-229.

Valenzuela, A. L. F. O. N. S. O., & Garrido, A. R. G. É. L. I. A. (1994). Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin. Biological research, 27, 105-105.

Visht, S., & Chaturvedi, S. (2012). Isolation of natural products. Current Pharma Research, 2(3), 584-599.

von Poser, G., Andrade, H. H., da Silva, K. V., Henriques, A. T., & Henriques, J. A. (1990). Genotoxic, mutagenic and recombinogenic effects of Rauwolfia alkaloids. Mutation Research, 232(1), 37-43.

Ward, F. M., & Daly, M. J. (1999). Hepatic disease. Clinical Pharmacy and Therapeutics (Walker R. and C. Edwards Eds.). Churchill Livingstone, New York, 195-212.

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