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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SCREENINGS OF DIFFERENT EXTRACTIONS FOR ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *BAUHINIA VARIEGATE* LEAF AGAINST PARACETAMOL INDUCED LIVER TOXICITY

Ashish Chourasia*, Love Kumar Soni

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India

**Corresponding author: ashish.davphd17@gmail.com*

ABSTRACT

The aim of present study was to investigate the effects of leaf extracts of *Bauhinia variegate* for the treatment of liver injury induced by the paracetamol. Dried and powdered materials of *Bauhinia variegate* leaf was extracted with ethanol and were subjected to phytochemical screening, later partitioned successively to obtain hexane, ethyl acetate and *n*-butanol partitions, respectively. All partitions were subjected to total phenolic content, total flavonoid content, *in vitro* antioxidant studies and hepatoprotective analysis. Following the hepatic injury induction, blood samples and liver were collected for the respective biochemical parameter and histopathological studies. Our results showed that ethyl acetate and *n*-butanol fractions exhibited a strong antioxidant activity *in vitro*. Ethyl acetate and *n*-butanol fractions scavenged DPPH radicals in a dose-dependent manner. *In vivo* histopathological studies indicated that paracetamol-induced liver injury was alleviated following ethyl acetate and *n*-butanol fractions treatment in rats. Ethyl acetate and *n*-butanol fractions significantly prevented paracetamol-induced elevations of alkaline phosphatase, glutamate pyruvate transaminase, aspartate aminotransferase, and total bilirubin levels in rats ($P < 0.05$). Moreover, isolated fractions of *Bauhinia variegate* leaf restored the decreased activities of hepatic antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzyme antioxidant glutathione, which were induced by paracetamol treatment. From these results it can be concluded that the *Bauhinia variegate* leaves extracts contain remarkable amount of flavonoids and can be used as reducing oxidative stress and hepatoprotective agent.

Keywords: Antioxidant, Hepatoprotective activity, *Bauhinia variegate,* Paracetamol, Liver toxicity, Flavonoids.

1. INTRODUCTION

Liver is the most important largest organ in the body. Its role in the regulation of various physiological processes, and its activity is associated with its vigorous functions, such as metabolism, secretion, and storage. It has capacity to detoxify waste products of metabolites and toxic substances as well as to create good compounds. One of the most widely used over-the-counter anti-pyretic and antianalgesic drug paracetamol has demonstrated several reports on its adverse effects on health [1]. Due to adverse effects on health, there is still a need to search for alternative new agents for the treatment of liver ailments with less or possibly no side effects, cheaper and widely available. Plants have been one of the good sources of obtaining new bioactive compounds [2, 3]. Hence, folkloric herbs with hepato-protective potentials to treat liver injury or disease have received considerable attention from researchers due mainly to their low toxicity and healing efficacy. Recently, several folkloric herbs have been investigated for their hepatoprotective role in experiments on animals.

Bauhinia variegata Linn is widely used in folklore medicine. Its leaves, bark, root, seeds and flowers are used for their medicinal properties. It has been used in ulcer, dyspepsia, bronchitis, leprosy, to prevent obesity, as an astringent, tonic, etc [4]. The *Bauhinia variegata* contains health promoting phenolic compounds, proteins, vitamin C and flavonoids, which have been shown good in vitro anti-oxidative and anti-inflammatory properties which are able to scavenge free radicals and protect the liver from carbon tetrachloride (CCl_4) injury [5].

Five flavonoids isolated from the different parts of *Bauhinia variegata* has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside. Phytochemical analysis of root bark of *Bauhinia variegata* Linn was reported to contain a new flavanone: (2S)-5, 7-

dimethoxy-3'-4'-methylene dioxyflavanone and a new dihydrobenzoxepin 5,6-dihydro-1,7dihydroxy-3,4-dimethoxy-2-methyldibenz (b,f) oxepin [6]. *Bauhinia variegata* Linn. stem is reported to have antitumour [7], anti-microbial [8], anti-inflammatory [9], hepato-protective [10], antioxidant and antihyperlipidemic [11] and immunomodulatory [12] activities.

Leaves of *Bauhinia variegata* Linn. is not taken up much for pharmacological evaluation. The aims of the study are to carry out biochemical and histological analyses to determine the effect of ethanolic extracts of leaves of *Bauhinia variegata* Linn. on paracetamol-induced injury in the liver's experimental rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Ethanol, hexane, ethyl acetate and *n*-butanol (Fisher Scientific), Paracetamol (PCM) and silymarin (Sigma-Aldrich) were used in the present study. α,α-Diphenyl-β-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich. The kits for determination of glutamate-pyruvic transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), total protein (TP), albumin (ALB), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione (GSH), malondialdehyde (MDA), and total bilirubin (TBIL) were purchased from local supplier. All other chemicals and reagents used were of analytical grade.

2.2. Collection of Plant Material

The leaves of *Bauhinia variegata* were collected in January 2020 from its natural habitat, Bhopal. A voucher specimen was identified by comparison with specimens available at the Herbarium of the Laboratory of Natural Products, Saifia College of Science, Bhopal MP, 462001, India. The leaves were dried under shade for 7 days at room temperature, segregated, and pulverized by mechanical grinder to form a coarse powder.

2.3. Animals

Albino Wistar male rats (125-175 g) were used for determination of hepatoprotective activity. The animals were housed in polypropylene cages at 25±1°C with the relative humidity of 55±5% under 12/12 h light/ dark cycles. They received standard food and water during experimentation. The food was withdrawn on the day before the experiment, but free access of water was allowed. A minimum of six animals was used in each group. The study protocol was approved by the Institutional Animal Ethics Committee, according to the regulation of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.4. Extraction of plant material

The dried and powdered material of leaves of *Bauhinia variegata* (500 g) was extracted with ethanol at room temperature with shaking and stirring. Combined ethanolic extracts were evaporated to dryness under reduced pressure below 40°C and then stored at (4°C) for further analysis.

2.4.1. Fractionation procedures

Ethanolic extracts were dissolved in distilled water and subjected to solvent-solvent fractionation. Ethanolic extract was fractionated with hexane, ethyl acetate and *n*-butanol in the order of their increasing polarity to obtain respective fractions [13].

2.4.2. Determination of percentage yield

The percentage yield was obtained using this formula W2-W1/W0× 100. Where, W2 is the weight of the fractions and the container, W1 the weight of the container alone and W0 the weight of the initial dried sample.

2.4.3. Phytochemical analysis

Methanol extract was analyzed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids [14, 15].

2.5. Aantioxidant activity

2.5.1. Determination of total phenolic content

The total phenolic content of the fractions was determined in triplicate according to the Folin-Ciocalteau spectrophotometric method [16], using gallic acid as a standard. The fractions were diluted in ethanol: water mixture at the concentration of 1:10 (v/v). An aliquot of 0.5 mL of the diluted sample was transferred to a test tube and, then, 2.5 mL of the Folin-Ciocalteau: water solution mixture (1:10, v/v) were added. The mixtures were vortexed followed by rest, in room temperature, for five minutes. After that, 2.0 mL of the sodium carbonate 4% (m/v) solution were added, and the mixtures were agitated again and kept at rest for two hours, at room temperature and protected from the sun light. Absorbance was taken at 740 nm using a UV-1203 spectrophotometer (Shimadzu Corporation; Japan). The results were calculated using the standard

curve of gallic acid with known concentrations (2.5 to 50 μg mL^{-1}), and they were expressed as mg of gallic acid (GAE)/g.

2.5.2. *DPPH radical scavenging activity*

The antioxidant activity was determined following the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) method [17] with slight modifications. A solution composed of 0.5 mL of the fractions were diluted in an ethanol solution 80%, 3.0 mL of ethanol 99%, and 0.3 mL of the DPPH radical 0.5 mM, diluted in ethanol: water solution mixture (80:20 v/v), were added to a test tube. A blank sample was prepared substituting the extract volume for an equal volume of ethanol 99%. After that, test tubes were agitated and incubated for 45 min at room temperature and protected from sunlight. The absorptivity readings were performed using a spectrophotometer (Shimadzu, model UV 1203) at 515 nm. The antioxidant activity results were expressed as μmol AA g^{-1} of fruit. Ascorbic acid (0.1 μmol) was used as a standard to construct the calibration curve and all tests were carried out in triplicate.

2.6. *In vivo* hepatoprotective activity

The approved IAEC method was adopted for screening of hepatoprotective activity. Fractions were suspended in 1% CMC solution before oral administration to animals. Experimental animals were divided into five groups of six rats each were used for the study. Group 1 received normal saline (1 ml/kg orally) for seven days. Group 2 were treated by 2% gum acacia + PCM (2 g/kg) for seven days. Group 3 received 100 mg/kg body weight dose of silymarin for seven days and Group 4 received 100 mg/kg body weight dose of ethyl acetate fraction and Group 5 received 100 mg/kg body weight dose of *n*-butanol fraction of *B. variegata* orally, once a day for seven days. On the eight day, after the administration of the respective treatments except group 1, all the animals in groups 2, 3, 4 and 5 were administered paracetamol 2 g/kg orally. After 24 hours, the blood samples were collected *via* orbital sinus puncture for the estimation of biochemical marker enzymes. Then the liver was carefully isolated and cleaned off extraneous tissue and preserved in 10% neutral formalin and then subjected to histopathological studies [18, 19].

2.6.1. *Assessment of biochemical parameters of liver*

The enzymatic parameters of serum like alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB), total protein (TP), and total bilirubin (TB) were assayed according to standard methods [20].

2.7. Statistical analysis

The outcomes were shown as mean±S.E.M. ($n = 6$). Statistical significance was determined by one-way analysis of variance with $p < 0.01$ and $p < 0.05$ considered significant followed by Dunnett multiple comparisons test. The analysis was performed by using Graph Pad InStat software.

3. RESULTS AND DISCUSSION

The leaf extracts of *Bauhinia variegate* was extracted with 80% ethanol by using cold maceration technique and further fractionated by using solvents of different polarities (hexane, ethyl acetate and n-butanol). The solvent fractions were investigated for their hepato-protective activity.

The percent yield of ethanol crude extract of *Bauhinia variegate* leaf was found to be 18.9%. The phytochemical tests were performed for the estimation of alkaloids, glycosides, flavonoids, and tannins in leaf extract and resulted in the presence of carbohydrates, proteins, alkaloid, saponins, flavonoids and phenolic compounds and results are given in table 1.

The extracts were dark brown residue, and there were compatible with the positive results of phytochemical analysis. The dark brown color may be due to the presence of large amounts of poly-phenolic compounds and flavonoids [21, 22]. The presence of various chemical constituents in *Bauhinia variegate* may be a potential cause of treatment of various disorders. The quality of the plant can be estimated by determining the physical parameters. These investigations are of great importance for carrying out the revalidation and estimation of its other pharmacological activities.

Ethanolic extract was fractionated with hexane, ethyl acetate and *n*-butanol in the order of their increasing polarity to obtain respective fractions. Each fraction was concentrated to dryness under reduced pressure and below (40-50°C) on a rotary evaporator to give ethyl acetate fraction [yield 9.2%, w/w] and *n*-butanol fraction [yield 8.4%, w/w] of *Bauhinia variegate*.

The total polyphenol content and antioxidant activity of the ethyl acetate and *n*-butanol fractions are shown in Table 2.

The ethyl acetate fraction of *Bauhinia variegate* had a total polyphenol content approximately two times that of *n*-butanol fraction. The same behavior was observed

in the DPPH assay. Total phenolics content of ethyl acetate and *n*-butanol fraction was found to be 40.28 ± 0.04 mg GAE 100 g^{-1} and 19.35±0.02 mg GAE 100 g^{-1} respectively. The DPPH antioxidant activities of ethyl acetate and *n*-butanol fraction was found to be 134.35±0.03 µmol AA g^{-1} of dry matter and 76.48±0.03 µmol AA g^{-1} of dry matter respectively.

Table 1: Phytochemical screening of leaf extracts of *Bauhinia variegate*

S. No.	Chemical Tests	A	B	C	D	Observation
1.	**Alkaloids**					
	Hager's reagent	-	-	+	-	Yellow coloured precipitate
	Dragendorff's reagent	-	-	+	-	Reddish coloured precipitate
2.	**Glycosides**					
	Legal's test	-	-	+	+	Pink to blood red colour
3.	**Phenols/Tannins**					
	Ferric chloride	-	-	+	-	Bluish black coloured
4.	**Flavonoids**					
	Lead acetate test	-	-	+	-	Yellow Coloured precipitate
	Alkaline reagent test	+	-	+	+	Colourless
5.	**Saponins**					
	Foam test	-	-	+	+	Layer of foam
6.	**Carbohydrates**					
	Fehling's solution test	+	+	+	+	Red coloured
7.	**Protein Amino acids**					
	Xantoprotein Test	-	-	+	+	Yellow coloured

A=Petoleum ether, B=Ethyl acetate, C=Ethanol and D=Water

Table 2: Total phenolic content and antioxidant activity of *Bauhinia variegate*

Bauhinia variegate	Total phenolic content	Antioxidant activity
		DPPH Assay
Ethyl acetate fraction	40.28±0.04	134.35±0.03
n-butanol fraction	19.35±0.02	76.48±0.03

Total phenolic content are expressed as gallic acid equivalent (mg GAE g^{-1} dry matter) and antioxidant activity are expressed as ascorbic acid equivalent (µmol AA g^{-1} dry matter), All values are expressed as mean ± SEM

Total phenolic content values were influenced by the non-polar to polar solvent extractions. Based on the solvent polarity chart, ethyl acetate is classified as an intermediate solvent, hence is able to extract inter-mediate-polarity phenolic compounds, while *n*-butanol and hexane extract polar and non-polar compounds, respectively. The present findings were further supported by Azlim Almey et al. [23], who suggested that higher extraction yields of total extractable polyphenols and total soluble solids are collected as the solvent polarity increases.

In discussing the relationship between total phenolic content value and antioxidant activity, it is worth to discuss on phenolic compounds in general. The most common plant phenolics include phenolics acids, flavonoids, tannins, with flavonoids being the most plentiful polyphenols in our diets. Polyphenolics, as antioxidants, are believed to have the capability to donate hydrogen to free radicals, consequently breaking the chain reaction of lipid peroxidation at the initiation stage [24].

3.1. *In vivo* hepatoprotective activity

3.1.1. Assessment of biochemical parameters of liver

PCM treated wistar rats showed significant increase in the serum levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB), and decrease in serum levels of total protein (TP) and albumin (ALB) ($P<0.05$). The treatment with silymarin, ethyl acetate fraction and n-butanol fraction significantly reduced the levels of ALT, AST, ALP, and TB ($P<0.05$). The 100 mg/kg of ethyl acetate fraction was more effective on the levels of ALT, AST, ALP and TB than n-butanol fraction. The outcomes of the results are tabulated in table 3.

Table 3: *In vivo* hepatoprotective activity of flower extracts of *Bauhinia variegate*

Indexes	Control Group	PCM Group	Silymarin Group	Ethyl acetate fraction	*n*-butanol fraction
ALP(IU/L)	64.90±1.79	187.24±3.12[a]	93.57±1.57[b]	93.57±1.57[b]	93.57±1.57[b]
AST(IU/L)	49.05±2.24	162.62±2.68[a]	99.32±0.90[b]	103.31±0.91[b]	101.06±0.92[b]
ALT(IU/L)	35.67±2.61	112.68±1.23[a]	44.84±0.61 [b]	48.29±0.71[b]	55.18±0.77[b]
ALB(g/L)	4.56±0.25	2.49±0.21[a]	3.99±0.11[b]	3.67±0.14[b]	2.84±0.14[ab]
TB(mg/dL)	0.41±0.03	1.98±0.26[a]	0.65±0.27[b]	0.81±0.15[b]	0.90±0.10[b]
TP(mg/L)	94.71±2.35	17.85±0.98[a]	81.7±1.12[b]	66.40±1.28[ab]	51.68±1.95[ab]

The values represent the mean ± SD, with n = 6 animals in each, [a]= P<0.05 compared with control, [b]= P<0.05 compared with negative control group

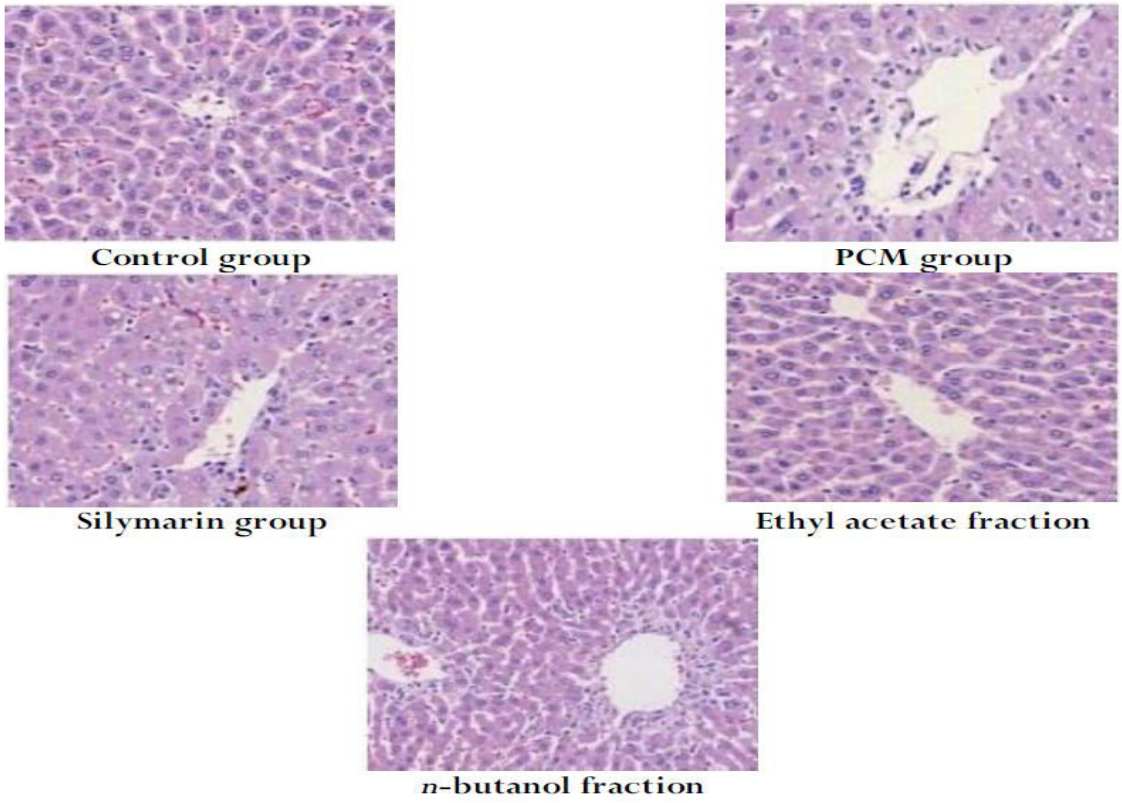

Fig. 1: Histopathological changes in the liver of rats with PCM-induced liver damage

Histopathological examination of the liver sections or wistar rats confirmed that the normal liver architecture was damaged with paracetamol administration. Livers of wistar rats treated with paracetamol appeared degeneration in hepatocytes, hepatic cell injury, focal necrosis, congestion in central vein and vascular swelling. However, pretreatment of ethyl acetate fraction and n-butanol fraction of *Bauhinia variegate* leaf at 100 mg/kg dose, significantly reduced the severity of histopathological injury (compared with the negative control group). The results of the biochemical tests and histopathological observations suggested that 100 mg/kg of each fraction is effective against liver toxicity. Thus, both fraction can be considered as prominent on paracetamol-induced liver damage.

Paracetamol is a known antipyretic and an analgesic which produces hepatic necrosis in high doses. It is usually eliminated mainly as sulfate and glucuronide. At toxic doses, the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive N acetyl-p-benzoquinone imine (NAPQI), by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage [25-27]. Free radical mediated process had been implicated in pathogenesis of most diseases. The protective effect of *Bauhinia variegate* on paracetamol induced hepato-toxicity in rats appears to be related to inhibition of lipid

peroxidation and increased levels of antioxidant enzyme in addition to free radicals scavenging action. Preliminary phytochemical studies revealed the presence of flavonoids in ethanolic extract of *Bauhinia variegates* leafs. Flavonoids are hepatoprotectives [28]. The observed antioxidant and hepatoprotective activity of *Bauhinia variegate* leafs may be attributed to the presence of flavonoids. Further studies to characterise the active principles and to elucidate the mechanism are in progress.

4. CONCLUSION

Bauhinia variegate has differentiated pharmacological prospective and was used since ancient times. It has a strong future in the field of herbal medicine, scientific research is requisite to discover the pharmacological impending of the plant. The hepatoprotective and antioxidant activities of ethyl acetate and n-butanol fraction could be linked to the presence of several flavonoid-based bioactive compounds. Moreover, these flavonoids might synergistically act with several saponins and tannins, detected during the phytocontituents screening, to exert the hepatoprotective and antioxidant activities. From the study, we can conclude that the ethanolic extract of *Bauhinia variegate*, has shown the ability to maintain the normal functional status of the liver and could be one of the herbal remedies for liver ailment especially for paracetamol induced hepatotoxicity.

5. ACKNOWLEDGEMENT

Authors are grateful to Professors and Management, School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India for support and institutional facilities. We declare that we have no conflict of interest.

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