Ethnopharmacological based Evaluation of Anogeissus pendula Edgew Extracts for Antioxidant and Hepatoprotective Potential

Abstract

Background: Anogeissus pendula has various reported ethnomedicinal uses and is reported to contain phenolic compounds which have antioxidant potential. Aim: The present study was undertaken to evaluate the in vitro antioxidant potential and in vivo hepatoprotective activity along with the oxidative stress parameters of stem bark and leaves of Anogeissus pendula for the first time. Settings and Design: Albino rats were divided into seven groups of six animals each. Healthy control (Group I) and toxic control (Group II) received the vehicle. Group III, IV, V, VI and VII were treated with silymarin (100 mg/kg body weight, orally) and two hydro-alcoholic extracts i.e., APB (stem bark) and APL (leaves) at doses of 200 and 400 mg/kg b. w., orally, respectively. Hepatotoxicity was induced by allyl alcohol. Materials and Methods: Albino Wistar rats of either sex between 8-12 weeks old were used. The plant parts were collected from Sawai Madhopur (Rajasthan, India) and extracted with hydro-alcoholic solvent to get two extracts i.e., APB (stem bark) and APL (leaves) which were investigated for the in vitro antioxidant potential through DPPH radical and \( \text{H}_2\text{O}_2 \) scavenging assay along with in vivo hepatoprotective potential through allyl alcohol induced hepatotoxicity. Statistical Analysis: Statistical comparisons between different groups were done by using one-way ANOVA followed by the Bonferroni test. \( P < 0.05 \) was considered significant. Results and Conclusions: APB showed more potent activity than APL in case of in vitro antioxidant potential with \( IC_{50} \) of 44.29 \( \mu \)g/ml in DPPH radical scavenging activity and 53.09 \( \mu \)g/ml in hydrogen peroxide scavenging assay. Both the extracts revealed antioxidant and hepatoprotective potentials in a dose dependent manner but more significant results were obtained in case of APB at 400 mg/kg. More amounts of phytoconstituents might be the reason behind the more significant activity of extract of stem bark than that of the leaves.

Keywords: Allyl alcohol, Anogeissus pendula, antioxidant, hepatoprotective

Introduction

Liver is the major organ involved in metabolism, detoxification and excretion. Its physiological activity results in the generation of highly reactive free radicals which produce toxicity by protein oxidation, enzyme inactivation and can bond covalently with membrane lipids causing lipid peroxidation which damage the liver tissue. Natural antioxidants such as glutathione, superoxide dismutase (SOD) and others provide protection against free radicals. Overproduction of free radicals in comparison to antioxidants results in organ damage. Therefore, strengthening of natural antioxidants or administration of exogenous antioxidants may be useful for protection. Inspite of the advancement in biomedicines, search is still on for hepatoprotective agents. Plants can easily serve this purpose due to their diversity of phytoconstituents. Ethnomedicinally as well as in traditional medicine systems in India, many medicinal plants and their formulations are claimed to be useful for liver disorders. Literature supports the view that the antioxidant activity is well associated with hepatoprotective activity.\(^\[1,2\]\)

With reference to the above, Anogeissus pendula which belongs to the family Combretaceae was selected. It is a family of 20 genera in which the plants are usually rich in tannin and Anogeissus genus is one of them.\(^\[3\]\) Anogeissus genus has reported ethnomedicinal significance in Asia and Africa.\(^\[4\]\) Anogeissus pendula Edgew (local name: ‘dhok’ or ‘kardhi’; English: ‘button tree’) grows in dry and mixed forests of India as a gregarious shrub or small tree.\(^\[5,6\]\) Different parts of the plant

Access this article online
Website: www.ancientscienceoflife.org
DOI: 10.4103/asl.ASL_219_16
Quick Response Code:
such as stem bark, seed, fruit and twig have been reported to be used for various ethnomedicinal uses in gastric disorder, wound healing, skin diseases, diarrhoea, dysentery, cough and burns.[4] The presence of alkaloids and phenolic compounds such as flavonoids, lignins and tannins have been reported in seeds, leaves and fruits.[7-9] It is well known that phenolic compounds are good antioxidants. The present study, for the first time, evaluates the hepatoprotective activity along with the in vitro and in vivo antioxidant activity of the hydroalcoholic extracts of stem bark (APB) and leaves (APL) on the basis of its traditional use in gastric disorder and presence of phenolic compounds.

Materials and Methods

Plant material and extraction

The plant parts were collected from Sawai Madhopur (Rajasthan, India) in the month of November. The plant was identified and authenticated by Dr. Krishnendra Singh Nama, Lecturer, Botany, Maa Bharti P.G. College, Kota (India). Collected plant parts were shade dried and powdered coarsely. 40 g of plant parts’ powder (stem bark and leaves) was preliminary defatted with chloroform and n-hexane, respectively. By using a Soxhlet apparatus further extraction was done with the hydro-alcoholic solvent. The extracts so obtained (APB-extract of stem bark; APL-extract of leaves) were concentrated under reduced pressure and freeze-dried to obtain the extracts which were stored in sealed vials in a freezer until tested.

Chemicals

1,1-diphenyl-2-picryl hydrazyl (DPPH), methanol-Hi-Media; ascorbic acid- Rankem; thiobarbituric acid (TBA), malondialdehyde (MDA-) Sigma-Aldrich; 5,5'-dithiobis-(2-nitro benzoic acid) (DTNB, Ellman’s reagent)- Thermo Fisher; sodium dodecyl sulphate (SDS)-SD Fine Chemicals; allyl alcohol- Qualikems and Silymarin was obtained from Meridian Medicare as gift sample. All other chemicals and reagents used were of analytical grade. Biochemical kits were of Span diagnostics and Merck. Double beam UV spectrophotometer was of Shimadzu; biochemical auto-analyser was of Labtronics; rotary evaporator was of IKA and freeze drier was of Telstar LyoQuest.

Animals

Albino Wistar rats (200 ± 25 g) of either sex between 8-12 weeks old were used. Animals were maintained under standard conditions (CPCSEA) and housed in separate cages under controlled conditions of temperature (22 ± 2°C), humidity (30-70%), 12 hours light–dark cycle and acclimatised for a week prior to dosing. All animals were given standard diet (Golden Feed, New Delhi) and water ad libitum. The study protocol was approved by Institutional Animal Ethics Committee (PBRI/IAEC/PN-467).

In vitro antioxidant activity

Antioxidant activity was evaluated through DPPH radical scavenging activity[10] and hydrogen peroxide scavenging assay.[11] In both, ascorbic acid was used as positive control.

Allyl alcohol induced hepatotoxicity

Hepatoprotective activity was evaluated by allyl alcohol induced liver hepatotoxicity[12] but with a few modifications. Albino rats were divided into seven groups of six animals each. Healthy control (Group I) and toxic control (Group II) received the vehicle (normal saline, 1 ml/kg body weight, orally). Group III, IV, V and VII were treated with silymarin (100 mg/kg body weight, orally), APL and APB at dose level of 200 and 400 mg/kg body weight, orally, respectively. On first and second day, after 7 hours of fasting, rats were treated accordingly. On second day, one hour later, the animals in groups II, III, IV, V, VI and VII were dosed orally with 0.4 ml/kg of 1.25% solution of allyl alcohol in double distilled water. Also, on second day after 16 hours of previous dosing treatment, animals were treated again. On third day, after 24 hours, the animals were anesthetised with anaesthetic ether after which the blood was collected from the retro orbital plexus of the eyes. Serum was separated from the blood samples. The clear serum obtained was used for the estimation of liver functional tests that were serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin using commercially available kits. The animals were sacrificed by cervical dislocation later and livers were excised, washed with ice cold normal saline followed by 0.15 M trisHCl (pH 7.4) and some part was fixed for histopathology which was carried out as per standard procedure. Remaining liver was used to make 10% homogenates in 0.15 M trisHCl (pH 7.4) for the estimation of lipid peroxidation[13] and superoxide dismutase[14] and in 0.1 M phosphate buffer (pH 7.4) for reduced glutathione.[15] Optical density was measured at 532 nm, 560 nm and 412 nm for lipid peroxidation, superoxide dismutase and reduced glutathione, respectively.

Statistical analysis

Statistical analysis was carried out using Sigma stat 3.5. Statistical comparisons between different groups were done by using one-way analysis of variance (ANOVA) followed by the Bonferroni test. Results were expressed as mean ± standard deviation (SD). P < 0.05 was considered significant.

Results and Discussion

In vitro antioxidant activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most
other free radicals. The delocalisation also gives rise to a deep violet colour. It is accepted as a well-known tool for evaluating antioxidant activity. The basis of the method is when a methanolic solution of DPPH is mixed with that of a substance that can donate a hydrogen atom such as an antioxidant it gives rise to the yellow-coloured reduced form i.e., diphenylpicrylhydrazine.

In the present study, decrease in the absorbance was used to calculate the percentage inhibition (I) of free radical in the presence of different concentrations of extracts and standard. Antioxidant activity was quantified by calculating the IC$_{50}$, which is the concentration of the extracts required to decrease the absorbance of free radical (DPPH) by 50%. Lower the value of IC$_{50}$ greater the free radical scavenging activity of the extract.

Both the extracts showed good concentration dependent free radical scavenging activity [Table 1], which supports the antioxidant activity of phenolic compounds whose presence was confirmed by qualitative investigation (unpublished results). Between the two extracts, APB showed more potent activity than APL with IC$_{50}$ of 44.29 µg/ml whereas APL had 71.42 µg/ml. This might be due to the presence of more phytoconstituents as evident from the quantitative investigation and chromatographic fingerprinting (unpublished results). However, scavenging activity of standard ascorbic acid was relatively more prominent than the extracts.

Hydrogen peroxide, a weak oxidizing agent can inactivate a few enzymes directly, frequently by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and once inside the cell it can probably react with Fe$^{2+}$ and possibly Cu$^{2+}$ ions to form hydroxyl radicals and react with biomolecules causing tissue damage and cell death. The antioxidants scavenge hydrogen peroxide and prevent cellular damage.

A behaviour similar to that of DPPH assay, was observed. Both extracts showed good concentration dependent radical scavenging activity. APB was found to be more potent with IC$_{50}$ of 53.09 µg/ml than APL which had 101.50 µg/ml. The presence of phenolic compounds supports antioxidant activity. Similar to that of DPPH assay, scavenging activity of standard ascorbic acid, was more prominent than the extracts.

**Allyl alcohol induced hepatotoxicity**

Hepatotoxicity produced by allyl alcohol is due to metabolism of the alcohol to acrolein.$^{[16]}$ Acrolein is a reactive $\alpha$$\beta$-unsaturated aldehyde, which is formed via direct oxidation by alcohol dehydrogenase and specially reacts with cellular GSH to form an aldehyde-GSH adduct which then is metabolized to the corresponding acid. In cells when depletion of GSH occurs, acrolein may react with essential macromolecules and lead to structural and functional derangement which eventually lead to irreversible injury.$^{[17]}$ In the early stages of allyl alcohol intoxication, a dramatic loss of hepatic glutathione (GSH) occurs which might be due to the alkylation of the thiol group of GSH by acrolein.$^{[18]}$ Allyl alcohol is known to produce periportal necrosis as the enzyme alcohol dehydrogenase is mainly localised in the periportal region which is mediated by metabolism of allyl alcohol to acrolein, glutathione depletion and lipid peroxidation. Acrolein has the ability to form covalent bonds with tissue proteins and binding to tissue macromolecules may be responsible for the hepatotoxic action of allyl alcohol.$^{[19,20]}$

Silymarin which is a purified extract from *Silybum marianum* is a known hepatoprotective agent due to its antioxidant potential. It contains flavonolignans and polyphenolic compounds. It is recognised as an effective liver remedy in Germany and in continental Europe. It is also used as a standard to evaluate other drugs as well. Hence, it is used in the present study to compare the extracts’ hepatoprotective activity.

In the present study, liver functional tests were carried out on serum samples. In toxic control, allyl alcohol induced hepatotoxicity caused a marked significant ($P < 0.001$) rise in SGPT, SGOT, ALP and total bilirubin in comparison to healthy control [Table 2]. Treatment with the extracts (APB and APL) at two doses (200 and 400 mg/kg) orally caused a marked decrease in the values of evaluated liver functional tests in comparison to toxic control but results

| Concentration (µg/ml) | 1,1-diphenyl-2-picryl hydrazyl scavenging activity | Hydrogen peroxide scavenging activity |
|----------------------|-----------------------------------------------|-------------------------------------|
|                      | DPPH: Percentage inhibition | H$_2$O$_2$: Percentage inhibition |
|                      | APB | APL | Ascorbic acid | APB | APL | Ascorbic acid |
| 20                   | 42.78 | 24.87 | 52.86 | 40.43 | 27.52 | 49.35 |
| 40                   | 45.86 | 41.18 | 55.51 | 47.37 | 33.93 | 57.00 |
| 60                   | 54.81 | 47.33 | 58.30 | 52.54 | 39.98 | 60.63 |
| 80                   | 63.10 | 52.67 | 61.67 | 56.72 | 44.88 | 64.46 |
| 100                  | 77.67 | 60.43 | 68.58 | 62.33 | 48.71 | 69.40 |
| IC$_{50}$ (µg/ml)    | 44.29 | 71.42 | 10.19 | 53.09 | 101.50 | 17.34 |

DPPH: 1,1-Diphenyl-2-picryl hydrazyl, H$_2$O$_2$: Hydrogen peroxide, IC$_{50}$: Inhibitory concentration 50, APB: Hydro-alcoholic extract of stem bark, APL: Hydro-alcoholic extract of leaves
were more significant (*P < 0.001) at 400 mg/kg than at 200 mg/kg. Among APB and APL, APB was more effective. At 400 mg/kg, APB caused more significant (*P < 0.001) decrease in SGPT (63.86 ± 8.158 IU/L), SGOT (72.26 ± 8.798 IU/L), ALP (72.26 ± 8.798 IU/L) and total bilirubin (0.67 ± 0.089 mg/dl). Most prominent effect was produced by the standard silymarin in comparison to extracts.

Oxidative stress parameters were evaluated in the liver homogenates. Lipid peroxidation was significantly (*P < 0.001) raised whereas, superoxide dismutase and reduced glutathione were significantly (*P < 0.001) depleted in toxic control in comparison to healthy control [Table 3]. Similar pattern of results were observed as discussed above, in which more significant (*P < 0.001 or *P < 0.05) results were obtained at 400 mg/kg than at 200 mg/kg and in case of APB than APL, when compared to toxic control. APB at 400 mg/kg caused a more significant (*P < 0.05) decrease in LPO i.e., 33.72 ± 5.235 nM MDA/g wet tissue and significant rise in antioxidants like SOD (*P < 0.05) to 115.90 ± 14.301 U/g wet tissue, GSH (*P < 0.001) to 0.79 ± 0.093 nmol/g wet tissue. Silymarin, a known hepatoprotective agent produced the most prominent results. Histopathological study also supports the hepatoprotective activity of extracts [Figure 1]. Treatment with extracts showed liver cells with normal morphology.

Hepatoprotective potential of extracts is maintained by the antioxidant potential which is supported by the good amount of phytoconstituents as evident from the quantitative evaluation which revealed that APB and APL had good content of total phenolics as 183.6 ± 0.90 and 154.6 ± 0.72 mg GAE/g dried extract but the APB showed more amounts of phytoconstituents than the APL (Unpublished results). Therefore, APB showed more potent antioxidant and hepatoprotective activity. Hepatoprotective activity revealed by the extracts was dose dependent as 400 mg/kg was more potent than 200 mg/kg. Allyl alcohol shows hepatotoxicity due to a metabolite acrolein which forms in the presence of alcohol dehydrogenase. So, the hepatoprotective activity of extracts might be due to the inhibition of the enzyme alcohol dehydrogenase responsible for the conversion.

Conclusions

The traditional use of *Anogeissus pendula* in gastric disorder is supported by our findings which reveal antioxidant and hepatoprotective potential due to the presence of phenolic compounds. The present plant can be a source of hepatoprotective agents and can be useful for the traditional healers in the treatment of hepatotoxicity. But this needs further investigation. Standardization of extracts, isolation and identification of active compounds along with further studies are required to understand the mechanism of action.

### Table 2: Effect of APB and APL on liver functional tests in allyl alcohol induced hepatotoxicity (n=6)

| Group               | Liver functional test | Total bilirubin (mg/dl) |
|---------------------|-----------------------|-------------------------|
|                     | SGPT (IU/L)           | SGOT (IU/L)             | ALP (IU/L) |
| Healthy control     | 43.08±3.471           | 54.31±7.483             | 103.28±5.842 | 0.50±0.048 |
| Toxic control       | 98.38±5.833*          | 114.03±7.773*           | 210.28±10.095* | 1.31±0.081* |
| Silymarin (100 mg/kg) | 54.98±6.055*        | 62.9±6.646*             | 114.88±8.862* | 0.56±0.059* |
| APB (200 mg/kg)     | 82.23±5.339*          | 90.76±6.392*            | 170.93±10.572* | 1.03±0.066* |
| APB (400 mg/kg)     | 63.86±8.158*          | 72.26±8.798*            | 127.56±10.976* | 0.67±0.089* |
| APL (200 mg/kg)     | 84.13±6.718*          | 96.43±6.989*            | 171.19±3.491* | 1.11±0.063* |
| APL (400 mg/kg)     | 71.18±8.011*          | 80.81±8.175*            | 140.80±8.357* | 0.74±0.067* |

Values are expressed as mean±SD, *P<0.001 in comparison to healthy control, *P<0.001, *P<0.05 in comparison to the toxic control and values are considered significant. APB: Hydro-alcoholic extract of stem bark, APL: Hydro-alcoholic extract of leaves, SD: Standard deviation, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase.

### Table 3: Effect of APB and APL on oxidative stress parameters in allyl alcohol induced hepatotoxicity (n=6)

| Group               | Oxidative stress parameters |
|---------------------|-----------------------------|
|                     | LPO (nM MDA/g wet tissue) | SOD (U/g wet tissue) | GSH (nM/g wet tissue) |
| Healthy control     | 22.88±4.762                | 145.45±25.753       | 0.96±0.110            |
| Toxic control       | 49.55±7.558*               | 65.90±22.268*       | 0.33±0.067*           |
| Silymarin (100 mg/kg) | 26.77±4.138               | 126.14±12.755*      | 0.87±0.136*           |
| APB (200 mg/kg)     | 43.72±7.722 (NS)          | 94.31±19.484 (NS)   | 0.62±0.082*           |
| APB (400 mg/kg)     | 33.72±5.235b              | 115.90±14.301b      | 0.79±0.093b           |
| APL (200 mg/kg)     | 45.53±1.821 (NS)          | 90.23±1.809 (NS)    | 0.53±0.038b           |
| APL (400 mg/kg)     | 40.66±6.561b              | 102.27±14.301b      | 0.74±0.091*           |

Values are expressed as mean±SD, *P<0.001 in comparison to healthy control, *P<0.001, *P<0.05 in comparison to the toxic control and values are considered significant. NS: Not significant, SD: Standard deviation, APB: Hydro-alcoholic extract of stem bark, APL: Hydro-alcoholic extract of leaves, LPO: Lipid peroxidation, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione.
Figure 1: Effect of APB and APL on histopathological damages induced by allyl alcohol in rats (H and E staining, ×40), (a) healthy control showing normal liver architecture; (b) toxic control showing necrotic lesions, vacuolisation of cytoplasm; (c) standard i.e., silymarin (d), (e) APB (200 and 400 mg/kg) and (f and g) APL (200 and 400 mg/kg) treatment showing normal hepatocytes with preserved cytoplasm and cellular boundaries in case of higher doses of extracts

Acknowledgment

Authors are highly thankful to Dr. Kiran Chaudhary, Botanist, Maa Bharti PG. College, Kota, India for helping in the collection of the investigated plant. Also to Dr. R. K. Dhawan, Principal-Director, Khalsa College of Pharmacy, Amritsar, India and S. Rajinder Mohan Singh Chima, Honorary Secretary, Khalsa College Charitable Society, Amritsar, India for providing the facilities to conduct research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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