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ORIGINAL ARTICLE

Cerebroprotective Actions of *Triticum aestivum Linn* Powder and *Bauhinia purpurea* Flower Powder in Surgically Induced Cerebral Infraction in Rats

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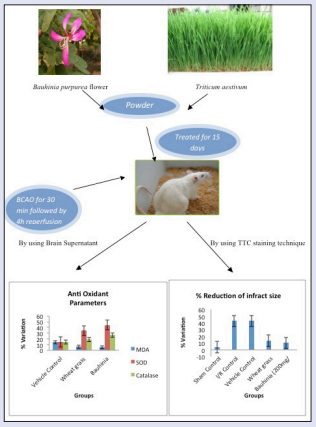
ABSTRACT

Objective: The prime objective of this study is to evaluate the cerebroprotective actions of *Triticum aestivum* (wheatgrass) powder and *Bauhinia purpurea* flower (dev kanchan) powder against the experimentally induced global ischemia reperfusion injury in rats. **Materials and Methods:** In the first phase of the studies, 1 h before the surgical procedure, the Wistar rats were orally served with varied doses of wheatgrass powder (5, 10, 30, and 100 µg/kg) and Bauhinia flower powder (30, 100, 200, and 300 µg/kg), respectively. The ischemia was induced by 30-min bilateral carotid artery occlusion in succession to reperfusion for 4 h. It was proved that the wheatgrass powder and Bauhinia flower powder yielded a significant, dose-dependent cerebroprotection in terms of reduction in cerebral infarct size when compared with the control group. Coming to the second phase of the studies, a certain potential dose of 10 µg/kg of wheatgrass and 200 µg/kg of Bauhinia flower powders was selected keeping the protective action in view, and the animals were treated for 15 days. **Results:** The major findings of the study are that wheatgrass and Bauhinia flower powders significantly augmented the magnitude of the antioxidant enzymes, viz., super oxide dismutase and catalase, and further reduced the levels of lipid peroxidation. **Conclusions:** The present study clearly showed that the wheatgrass powder and Bauhinia flower powder possess significant antioxidant properties that may act as a key ingredient in various ayurvedic preparations for the treatment of various diseases like cerebral ischemic reperfusion injury.

Key words: *Bauhinia purpurea*, catalase, ischemia reperfusion injury, MDA, *Triticum aestivum Linn*, SOD

SUMMARY

- The wheat grass contains high amount of bioflavonoids, alkaloids, SOD etc which are responsible for anti oxidant activity.
- The *Bauhinia purpurea* contains glycosides, flavonoids and also plays a major role in DPPH activity which is responsible for anti oxidant activity.
- The wheat grass (10 mg/kg) and bauhinia (200 mg/kg) significantly ($P < 0.0001$) reduced the percentage of infract size when compared to Ischemia reperfusion control group.
- The wheat grass (10 mg/kg) and bauhinia (200 mg/kg) significantly ($P<0.0001$) reduced the lipid peroxidation (MDA) and increased SOD and Catalase.



Abbreviations used: BCAO: Bilateral Carotid Artery Occlusion, MCA: middle cerebral artery, ROS: reactive oxygen species, SCMC: Sodium carboxy methyl cellulose, p.o: Per oral route, T.T.C: Triphenyl tetrazolium chloride, MDA: Malondialdehyde, SOD: Super oxide dismutase.

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INTRODUCTION

A recent survey revealed that the stroke is the second leading cause of mortality worldwide. Stroke, a sudden change in the blood supply to cerebral hemisphere, is a serious medical condition in which the brain does not get enough blood supply.[1] The brain, the most sensitive tissue, requires one-fourth of total oxygen supply. Any amount of altercation to this supply chain leads to ischemia/blockage of blood provision to cerebral neurons causing complex chain reaction that ultimately culminates in cellular death.[2] The pathophysiological mechanisms behind the ischemic cascade involve in manifold dysfunctions such as energy failure, excitotoxicity, cortical spreading depression, blood–brain barrier distraction, and apoptosis.[3] Moving further, the brain, indeed, is susceptible to oxidative stress due to its high oxygen consumption, abundant unsaturated fatty acids, and low levels of endogenous antioxidants, although the oxidative stress has a destructive effect on the pathophysiology of ischemia reperfusion injury.[4]

Ischemia, in a broader sense, comprises focal (occlusion of middle cerebral artery), global ischemia (occlusion of bilateral carotid artery), and intraparenchymal hemorrhage.[5] The free radicals and cellular death are the major contributors to the pathogenesis of ischemic reperfusion injury. All in all, ischemia increases lipid peroxidation (MDA), an autocatalytic mechanism leading to oxidative destruction of cell

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membranes, and reactive oxygen species (ROS), which cause secondary neural tissue damage.

Triticum aestivum (wheatgrass) is a nutraceuticals that comes under the family of Poaceae. Wheatgrass is fiber in nature and rich in vitamins, viz., A, C, E, and K. It contains high amount of bioflavonoids such as apigenin, quercetin, luteoline, and 70% chlorophyll. It also incorporates alkaloids, tannins, saponins, sterols,[6] and pharmacological active enzymes including cytochrome oxidase transhydrogenase, super oxide dismutase (SOD) and amino acids, which are responsible for various pharmacological activities.[7] It, likewise, demonstrates anticancer activity, antiulcer activity, antioxidant activity, antiarthritic activity, and blood forming activity.[8] Hitherto, the above-mentioned details provided plenty of ammunition to prove that wheatgrass will also exhibit cerebroprotective activity.

On the contrary, *Bauhinia purpurea* is a deciduous tree, which belongs to the family of Leguminosae. Its aerial parts contain glycosides, flavonoids, 6-butyl-3-hydroxy flavanone, amino acids, phenyl fatty acid esters lutine, and β-sitosterol. The ethanolic extract of bauhinia exhibits analgesic, anti-inflammatory, antioxidant, hepatoprotective, and antiulcer activities.[9] The bauhinia flowers contain high amount of flavonoids, which are responsible for the hypoglycemic and cardiotonic activities.[10]

Nowadays natural products are gaining potential importance than the pharmaceutical products because of their tremendous activities and less side effects. Much of the research is focused on the plant-derived drugs; therefore, the present research work made an attempt to evaluate the cerebroprotective potential of wheatgrass and Bauhinia flowers based on the antioxidant properties.

MATERIALS AND METHODS

Chemicals

- Thiopentone sodium (Neon-Labs-Mumbai).
- 2,3,4-tetrazolium chloride (TTC) (National Chemicals, Vadodara)
- Phosphate buffer solution (pH-7.4)
- Wheatgrass powder (self-prepared)
- Bauhinia flower powder (self-prepared)
- Sodium carboxy methyl cellulose (SCMC)

Preparation of extract

Wheatgrass was self-cultivated in the university surroundings, shade dried, and made into powder form and authenticated by the Botany Department of Andhra University, Visakhapatnam, India (voucher specimen AU/TA/03). The powder was suspended in 1% sodium carboxy methyl cellulose. Bauhinia flowers were collected in and around the university surroundings, shade dried, and made into powder form and authenticated by the Botany Department of Andhra University, Visakhapatnam, India (voucher specimen AU/BP/09). The powder was suspended in 1% SCMC.

Animals

Wistar albino rats of either sex weighing 150–200 g were used. The animals were maintained on 12-h light and dark cycles. They were fed standard diet and water *ad libitum*. The animal housing and handling were in accordance with CPCSEA guidelines. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC) bearing the registered No. 516/PO/c/01/IAEC.

Experimental design and treatment (first phase)

The animals were acclimatized for 1 week and randomly divided into 11 groups. Each group consists of six animals ($n = 6$).

Group 1—Sham control.

Group 2—Ischemia reperfusion control (I/R), received 0.2 mL of saline.

Group 3—Vehicle control, received 0.2 mL of SCMC 1 h before the surgical procedure (p.o.).

Group 4—Received wheatgrass 5 μg/kg 1 h before the surgical procedure (p.o.).

Group 5—Received wheatgrass 10 μg/kg 1 h before the surgical procedure (p.o.).

Group 6—Received wheatgrass 30 μg/kg 1 h before the surgical procedure (p.o.).

Group 7—Received wheatgrass 100 μg/kg 1 h before the surgical procedure (p.o.).

Group 8—Received bauhinia 30 μg/kg 1 h before the surgical procedure (p.o.).

Group 9—Received bauhinia 100 μg/kg 1 h before the surgical procedure (p.o.).

Group 10—Received bauhinia 200 μg/kg 1 h before the surgical procedure (p.o.).

Group 11—Received bauhinia 300 μg/kg 1 h before the surgical procedure (p.o.).

Induction of ischemia reperfusion injury

All the rats were anesthetized with thiopentone sodium (i.p., 30 μg/kg body weight) incubated and were placed in a supine position. The rats were maintained at 37°C under a bulb.

A small median incision was made in the neck and both carotid arteries were separated from vagus nerves, and then bilateral carotid arteries were exposed and occluded for 30 min using nylon thread. After ischemia induction, the occlusion was removed by releasing the knots and the animals were allowed to reperfusion for 4 h.[11]

Determination of ischemia infarct size

After reperfusion the animals were sacrificed by decapitation technique, brains were isolated, weighed, and frozen at 4°C for 5 min. The frozen brains were sliced (1–2 mm) and the sections were immersed in 1% TTC solution prepared in phosphate buffer (pH 7 .4) and incubated at 37°C for 20 min.[12] The TTC is converted to red formazone pigment by NAD and dehydrogenase present in living cells, hence the viable cells were stained deep red and the infarct cells have lost the enzymes and thus remain unstained. The infracted part of the brain was separated, weighed, and expressed as percentage (%) reduction of infarct size.[13] The results were given in Table 1 and percentage variation of infarct size is given in Figure 1.

Experimental design and treatment (second phase)

From an acute administration of different doses, an effective dose of 10 μg/kg wheatgrass and 200 μg/kg Bauhinia flower powders had been selected based on the efficiency of the dose and administered orally for 15 days.

Group 1–Vehicle control, received 10% SCMC

Group 2–Received 10 μg/kg wheatgrass powder

Group 3–Received 200 μg/kg Bauhinia flower powder

Preparation of brain supernatant

After 4-h reperfusion, the animals of all groups were sacrificed by decapitation and brains were isolated, washed, and subsequently blotted on filter paper. The brains were weighed and homogenized in 0.1 M cold phosphate buffer using homogenizer. The homogenate was cold centrifuged at 1000 RPM for 3 min, and the supernatant was divided into two portions, one portion of the supernatant was used for estimation of lipid peroxidation. The remaining portion was further centrifuged at 12,000 RPM for 15 min, and antioxidant parameters (SOD, catalase) were estimated.[14]

Determination of oxidative stress markers (MDA, SOD, and catalase levels)

These were used as an index for measuring the tissue damage induced by oxidative stress during the cerebral ischemic reperfusion injury. MDA levels were measured as described by Ohkawa *et al.*[15] The SOD levels were measured as described by Kakkar *et al.*[16] The catalase activity was measured as described by Medhi *et al.*[17] The results were given in Tables 2–4 and Figure 2.

Statistical analysis

The results were expressed as mean ± SEM. The percentage difference in infarct size, MDA, SOD, and catalase was analyzed by one-way ANOVA followed by Dunetes test were $P < 0.001$ were considered as statistically significant. Statistical analysis was performed by PRISM software (version 5.0).

RESULTS

The wheat grass (10 mg/kg) and bauhinia (200 mg/kg) powders showed a significant ($P < 0.001$) cerebroprotection when compared to the ischemia reperfusion control (I/R) group in terms of reduction in percent infract size [Table 1 and Figure 1] and in oxidative stress markers (MDA, SOD, Catalase).

DISCUSSION

Cerebral ischemic reperfusion injury is an acute inflammatory response that gets generated during the blockade of the carotid artery. As brain requires a huge amount of blood supply, even a minute fraction of blood blockage leads to complex chain reactions and causes the shutting down of neural activity. The brain consists of different neurotransmitters, in which the excitatory neurotransmitter glutamate is present at high concentrations. All the same, ischemia leads up to the production of huge concentration of glutamate, which in return activates NMDA and AMPA receptors leading to an influx of Na^+, Ca^+, and efflux of K^+ ions. In a nut shell, the whole process begets membrane shunting.[17] During ischemic conditions, along with a cytotoxic mechanism, some endogenous protective mechanisms prove themselves to be vital in enhancing the blood circulation through the ischemic neurons. These ischemic neurons, with the course of time, accrue the oxygen demand in surrounding tissues leading to damage of vascular and intraparenchymal tissues.[18]

It was evidenced that the natural products would show a significant dose dependent cerebroprotection, in terms of decrease in percentage of infarct size in the ischemia reperfusion controlled group when compared to the sham controlled group.[19] In the present study the wheat grass and the bauhinia showed the significant reduction in infract sizes which are in concordance with above statement.

The damage of vascular and parenchymal tissues of the brain during ischemia is further increased during reperfusion (recirculation) because the sudden flow of blood through the neurons increases the threshold of the neurons, which leads to over production of various mediators

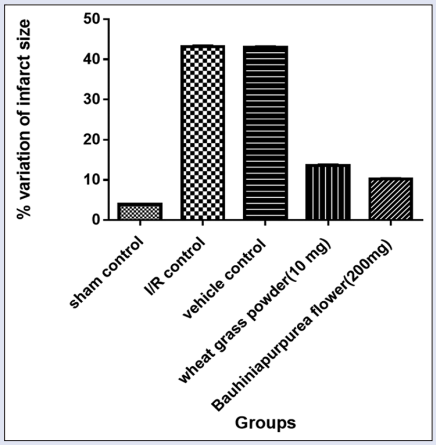

Figure 1: Percentage variation of wheat grass powder and *Bauhunia purpurea* flower powder on reduction of infract size in cerebral ischemia reperfusion injury in rats

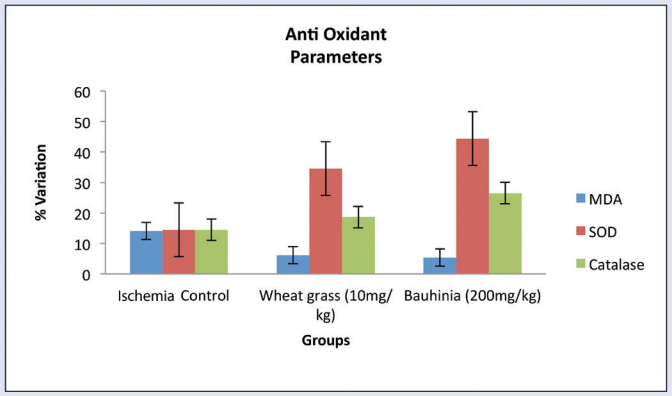

Figure 2: Percentage variation of wheat grass powder and *Bauhinia purpurea* flower powder on biochemical parameters (MDA, SOD, Catalase).

Table 1: Effect of wheat grass powder and *Bauhinia purpurea* flower powder on reduction of infracts size in cerebral ischemia reperfusion injury in rats.

Serial no	Sham control	Ischemia reperfusion control	Vehicle control	Wheat grass powder (10 mg/kg)	*Bauhinia purpurea* flower powder (200mg/kg)
1	3.54	42.92	43.26	13.52	10.02
2	4.08	43.18	43.12	13.64	10.25
3	3.84	43.06	43.07	13.80	10.24
4	3.92	43.08	42.82	13.92	10.06
5	4.16	43.87	43.15	12.94	10.50
6	3.75	43.12	43.02	13.62	10.44
Mean± S.E.M	**3.84±0.210**	**42.97±0.349*** **	**42.97±0.194*** **	**13.55±0.350*** **	**10.22±0.204*** **

The values are expressed as Mean±S.E.M (n=6) and P *** value <0.001 is considered as statistically significant.

Table 2: Effect of wheat grass powder and *Bauhinia purpurea* flower powder on percentage variation of MDA (nmol/g wet tissue) levels of infracted tissue in cerebral ischemia reperfusion injury in rats.

Serial no	Ischemia control	Wheat grass powder (10mg/kg)	*Bauhinia purpurea* flower powder (200mg/kg)
1	13.672	5.536	5.416
2	13.781	5.805	5.625
3	13.633	5.767	4.937
4	14.982	6.654	5.153
5	14.651	6.550	5.665
6	14.011	6.793	5.434
MEAN±SEM	**14.121±0.627**	**6.184±0.578*** **	**5.371±0.280*** **

The values are expressed as Mean±S.E.M (n=6) and P *** value <0.001 is considered as statistically significant.

Table 3: Effect of wheat grass powder and *Bauhinia purpurea* flower powder on percentage variation of SOD (U/mg protein) levels of infracted tissue in cerebral ischemia reperfusion injury in rats.

Serial no	Ischemia control	Wheat grass powder (10mg/kg)	*Bauhinia purpurea* flower powder (200mg/kg)
1	14.695	34.250	44.469
2	14.382	34.858	44.248
3	14.446	34.394	44.969
4	14.289	35.124	44.256
5	14.864	34.328	44.254
6	14.298	34.464	44.264
MEAN±SEM	**14.495±0.233**	**34.569±0.344*** **	**44.412±0.286*** **

The values are expressed as Mean±S.E.M (n=6) and P *** value < 0.001 is considered as statistically significant.

Table 4: Effect of wheat grass powder and *Bauhinia purpurea* flower powder on percentage variation of Catalase (μmoles/min per mg protein) levels of infracted tissue in cerebral ischemia reperfusion injury in rats.

Serial no	Ischemia control	Wheat grass powder (10mg/kg)	*Bauhinia purpurea* flower powder (200mg/kg)
1	12.970	18.674	25.858
2	13.524	18.019	25.940
3	13.118	18.412	27.295
4	13.038	18.695	26.945
5	12.987	18.254	25.980
6	12.820	18.982	27.104
MEAN±SEM	**13.076±0.24**	**18.636±0.324*** **	**26.520±0.412*** **

The values are expressed as Mean±S.E.M (n=6) and P *** value < 0.001 is considered as statistically significant.

such as ROS (superoxide, hydroxyl, and nitric oxide [NO] radicals) and catabolic enzymes (phospholipase A_2 and C [PLA_2 and PLC]).[20] It stands as evidence to the ROS that are elevated during ischemia and reperfusion.

Substantially,reperfusion plays a major role in the pathophysiology of ischemic stroke or ischemia reperfusion-related injury.[21] In the present study, it was proved that the wheatgrass powder (10 μg/kg) and Bauhinia flower powder (200 μg/kg) significantly decreased the levels of MDA and increased the levels of antioxidant enzymes like SOD and catalase in the infracted brain tissue of rats when compared with sham control.

There are no particular scientific evidences of wheatgrass and Bauhinia flower powder to lipid peroxidation mechanisms, but the antioxidant activity of wheatgrass[22] and DPPH free radical scavenging activity of Bauhinia flower[23] was reported. The wheatgrass also contains antioxidant vitamins like vitamin E, vitamin C, chlorophyll, β-carotene, and others.[24] The Bauhinia flower contains polyphenols, vitamin E, vitamin C, and flavonoids like quercetin and isoquercetin.[25,26] These are the known compounds having antioxidant activity. Although these compounds might be valuable in decreasing the lipid peroxidation caused due to oxidative stress during cerebral ischemia reperfusion injury.

SUMMARY

To evaluate the cerebroprotective activity, *in vivo* bilateral common carotid artery occlusion-induced cerebral ischemia reperfusion model (30 min ischemia and 4 h reperfusion) was chosen. After reperfusion, the cerebral damage was determined using TTC staining technique. In the first phase of treatment, a dose-dependent effect of wheatgrass powder (5, 10, 30, and 100 μg/kg) and Bauhinia flower powder (30, 100, 200, and 300 μg/kg) was carried out and a potential dose of wheat grass (10 μg/kg) and Bauhinia (200 μg/kg), based on the data, was selected, respectively. The powders were suspended in 1/% sodium carboxy methylcellulose and administered chronically for 15 days. On the 15th day, the ischemia was induced followed by reperfusion. The focal points were infarct size percentage and antioxidant role of wheatgrass and Bauhinia flower powders in cerebral ischemic reperfusion injury. Both wheatgrass and Bauhinia powders significantly decreased MDA levels and increased the SOD and catalase levels.

CONCLUSION

It is possible to demonstrate conclusively that the corporal mechanisms that involve in the cerebroprotective activities, viz., radical scavenging and antioxidant activity, will greatly be reverberated by both wheatgrass powder and Bauhinia flower powder. It needs to be further studied to explore the other possible mechanisms that may betide in a cerebroprotective activity of wheatgrass and Bauhinia flower powder.

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Conflicts of interest

There are no conflicts of interest.

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