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Research Article

# Effect of A Single Dose Adminstration of Wheat Bran Extract and Its Active Components On Acute Ischemicbrain Injury

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**ABSTRACT**
Three known compounds were isolated from wheat bran (Graminae), namely β-sitosterol 3-*O*- β, D-glucopyranoside (WB1), Sucrose (WB2) and 10(cis), 13(cis)-octadecadieneoic acid (WB3) for first time by successive column chromatography. The structures were determined mainly by spectroscopic method ($^1$H, $^{13}$C–NMR). This study aimed to evaluate antioxidant activity and determine the effect of ethanol extract of wheat bran and its active components on oxidative stress induced by cerebral ischemia-reperfusion injury (I/R) by occlusion of the left common carotid artery (CCA) in the rat. They restored the I/R-induced depletion of super oxide dismutase activity (SOD) and reduced glutathione (GSH) contents, with reduction of malondialdehyde (MDA) and nitric oxide (NO) contents that elevated during cerebral ischemia-reperfusion injury. In conclusion, WBI, WB3 and total ethanol Bran extract ameliorated the oxidative stress resulted from cerebral ischemia-reperfusion confirmed by histopathological examination and immunohistochemical analysis.

**Keywords:** Bran; Steroidal Saponin, unilateral carotid artery occlusion (CAO), Histopathology, immunohistochemistry, Cyclooxygenase-2 (Cox2).

## INTRODUCTION

Wheat bran, a by – product of flour milling, is composed of the pericarp and the outer most tissue of the seed including the aleurone layer[1], wheat rich in essential amino acids, minerals, vitamins, beneficial phytochemicals and dietary fiber components which contributes to the human diet[2]. Wheat bran is used as a source of dietary fiber for preventing colon disease, stomach cancer, breast cancer, gall bladder disease, hemorrhoids and hiatal hernia[3]. It is also used for treating constipation, irritable bowel syndrome, high cholesterol, high blood pressure and type's 2-diabetes[4,5].

*Preliminary phytochemical investigation*

Wheat bran was screened for the presence of carbohydrates[6] and/or glycosides, alkaloids and or nitrogenous base[7], saponins[6], anthraquinons[8], unsaturated sterols and/or triterpenes[9,10], coumarins[11], tannins[12] and flavonoids[13]. The phytochemical screening revealed the presence of high contents of flavonoids, saponins and carbohydrates, and absence of tannins, alkaloids, courmains and anthraquinones.

## EXPERIMENTAL

*General*

The NMR spectra were recorded at bruker NM spectrometer operating at (600-400MHz for $^1$H) and (100-125MH$_Z$ for $^{13}$C). All NMR spectra were obtained in DMSO-d$_6$, using TMS as internal standard, with the chemical shifts expressed in δ and coupling constants (*J*) in Hertz. For column chromatography, Sephadex LH-20 (pharmacia, Uppsala, Sweden), Silica gel 60-120 MESH (NICE chemicals Pvt. Ltd. India), were used. For paper chromatography Whatman paper No. 1 sheets (What man Ltd., England) were used, while silica gel G powder was used for Saponin CC and $F_{254}$ for TLC. (Merck, Germany).

*Plant material*

Wheat bran (Triticum vulgare L) used in the study was supplied by Haraz flour Milling, Cairo, Egypt. The bran was cleaned and stored in cool and dry place prior to use.

*Animals*

Male Wister albino rats, weighing 250-280g and Swiss mice weighing 25-30g were used throughout the experiments. The animals were obtained from the animal house colony of the National research centre, Dokki, Giza, Egypt. The animals were housed in standard metal cages in an air conditioned room at 22 ± 3˚C, 55 ± 5% humidity and provided with standard laboratory diet and water *ad libitum*. Experiments were performed between 9:00 and 15:00 h. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and the animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory animals[14].

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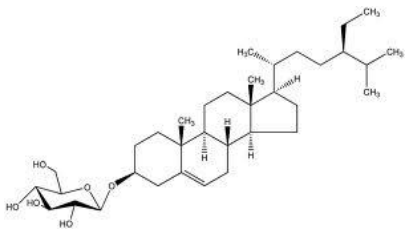
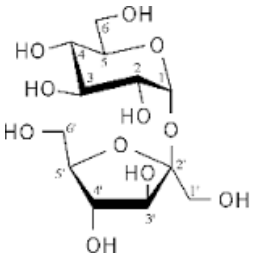
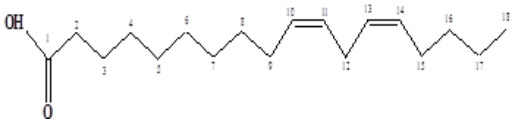


β-sitosterol-3-*O*-β-**D**-glucopyranoside



α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside



10(cis), 13(cis)-octadecadienoic acid

*Extraction and isolation*

The dried Bran powder (1 kg) were exhaustively extracted with 85% ethanol (3 x 1.5, and 3 x 1.25), respectively, under reflux (70ºC). After evaporation of the solvent, the concentrated residue was (69.7 g) The Wheat bran extract (WBE) was dissolved by water, and then extracted by butanol followed by ethyl acetate. Butanol fraction of wheat bran ethanolic extract (WBEB, 6g), ethyl acetate fraction of wheat bran ethanolic extract (WBEE, 10g). WBEE was selected for further research, which was adsorbed on silica gel and subjected to column chromatography over silica gel (60 cm x 25 mm, 200-300 mesh) using series of $CHCl_3/CH_3OH$ as a mobile phase. Twelve collective fractions were obtained, upon series of purification of these fractions over Sephadex $LH_{20}$ using ethanol as a mobile phase; three pure compounds (WB1, WB2 and WB3) were isolated.

*Structure elucidation*

Compound WB1: White crystal, soluble in a mixture of chloroform and methanol, $R_f$ value 0.50 (chloroform: methanol, 4:1 as mobile phase). It gave position stable violet ring with libermann burchard test indicating a triterpenoid and/or steroid skeleton. $^1$H- NMR (600 MHz, DMSO-$d_6$): δ 5.32 (m, 1H, H6), 4.21 (d, J = 7.8Hz, H-1'), 3.63 (tdd, H3), 3.37-3.46 remaining of sugar protons, 6-$CH_3$ at 1.23 (s, 3H, $CH_3$- 19), 0.95 (s, 3H, $CH_3$-18), 0.89 (d, 6H, $CH_3$–26, 27), 0.80 (s, 1H, $CH_3$-21) and 0.64 (s, 1H, $CH_3$–29). Compound WB1 was obtained through their spectral values from the ethanol extract of wheat bran, the $^1$H NMR spectrum showed downfield shift 1H, intensity at $\delta_H$ 5.32 ppm, indicative of olefinic proton (H–6)[15].The spectrum had a multiplet at $\delta_H$ 3.63 ppm indicative of an oxymethine proton (H-3)[15] the spectrum showed the presence of six methyl protons at $\delta_H$ 1.23 (H-19), 0.95 (H-18), 0.89 (H-26 and 27), 0.80 (H-21) and 0.64 (H-29) ; Respectively additionally one-β-anomeric protons was assigned at δH- 4.21 ppm (J= 7.8Hz) and the signals between $\delta_H$ 3.37-3.46 ppm typical for a sugar moiety. $^{13}$C NMR spectrum (table 1) revealed the presence of 35 carbon signals, of which 29 carbons were attributed to aglycone moiety and six to sugar moiety, the aglycone signals were at δc 140.44 (C–5), 41.86 (C–13) and 36.22 (C–10), were assigned to three quaternary carbons. The down field shift at δc 76.87 ppm (C–3) indicates the presence of *O–B*-sugar. Two olefinic carbon signals at δc 140.44 and 121.24 ppm were for (C-5 and C-6) and the carbon signals of the sugar moiety at δc 100.75 (C–1'), 76.76 (C3'-5' overlaped), 73.46 (C–2'), 70.09 (C-4') and 61.09 (C–6') were well consistent with those of glucose[16]. These data confirmed that compound $WB_1$ is a β-sitosterol-*3-O-B-D*-glucopyranoside. Compound WB2: Fine colorless, odorless crystalline powder with pleasing sweet taste, $R_f$ 0.39 (ethyl acetate: pyridine: water: acetic acid 6: 3: 1: 0.5). On the basis of its chromatographic properties compound WB2 was expected to be α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside. Signal multiplicities, chemical shifts in the $^1$H-$^{13}$C NMR spectra (table 2) of WB2, revealed the resonance of typical signals of two sugar units[17,18], a doublet signal with small coupling constant at $\delta_H$ 5.28 & $\delta_C$ 91.86 was assignable to the anomeric proton of the sugar unit. According to chemical shifts of this proton as well as the presence of oxymethylene proton at $\delta_H$ 3.77, $\delta_C$ 60.58, characteristic of $H_2$-6 of α-glucose unit. Furthermore, two oxymethylene proton and carbon signals at ($\delta_H$ 3.57, $\delta_C$ 62.14) and ($\delta_H$ 3.77 & $\delta_C$ 62.24) were attributed to $H_2$-1`and $H_2$-6` of fructose unit, respectively thus, two sugar units were determined as glucose and fructose. A comparison of the anomeric chemical shifts for fructose & glucose residues in WB2 and methyl α-and β-fructofuranoside and glucopyranoside[17-18] indicated that the ring of fructose is in the fructofuranoside and that it is β- linked to α-glucose. In addition to mild acid hydrolysis it gave glucose & fructose (1:1) this clearly suggested that this compound could be a disaccharide as these at made of two sugar moieties with six carbon atoms each and was in the line with the structure of sucrose which has two sugar units (glucose and fructose) joined by glycoside linkage between C-1 anomeric of glucose and C-2` (quaternary) fructose[19]. This conclusion was also clarified by comparing the spectral data of WB2 with previously investigation of the $^{13}$C- NMR characteristic for sucrose[17,18]. WB2 was confirmed as α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside. Compound WB3: This compound had a typical $^1$H NMR spectrum of a long chain fatty acid with two non-conjugated double bonds[20]. There were four olefinic protons (H–10, H–11, H-13, H-14) at 5.31-5.32 ppm, four allylic protons (H-9 and H-15) at 1.97 ppm, and two bis-allylic protons (H-12) at 2.49 ppm in the $^1$H-NMR spectrum (Table 3). The compound WB3 had a typical $^{13}$C–NMR spectrum of a long chain unsaturated fatty acid with six easily recognized signals at 174.51, 33.66, 23.24, 31.29, 22.09

Table 1: $^{13}C$ -NMR (DMSO-$d_6$, 600 MHz)

| C. No. | $\delta_C$ | C. No. | $\delta_C$ | C. No. | $\delta_C$ | C. No. | $\delta_C$ | C. No. | $\delta_C$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.83 | 8 | 31.37 | 15 | 23.86 | 22 | 33.33 | 29 | 11.79 |
| 2 | 29.26 | 9 | 49.59 | 16 | 27.80 | 23 | 25.40 | 1` | 100.75 |
| 3 | 76.87 | 10 | 36.22 | 17 | 55.41 | 24 | 45.12 | 2` | 73.46 |
| 4 | 40.03 | 11 | 20.59 | 18 | 11.68 | 25 | 28.68 | 3` | 76.76 |
| 5 | 140.44 | 12 | 38.29 | 19 | 19.11 | 26 | 18.93 | 4` | 70.09 |
| 6 | 121.24 | 13 | 41.86 | 20 | 35.48 | 27 | 19.73 | 5` | 76.76 |
| 7 | 31.42 | 14 | 56.17 | 21 | 18.62 | 28 | 22.59 | 6` | 61.09 |

Table 2: $^{1}H$ & $^{13}C$ NMR data for compound WB2

| | No of atoms | $\delta_H$ | $\delta_C$ |
|---|---|---|---|
| | 1 | 5.28 | 91.86 |
| | 2 | 3.54 | 71.73 |
| Glucose moiety | 3 | 3.78 | 72.98 |
| | 4 | 3.78 | 69.93 |
| | 5 | 3.56 | 72.91 |
| | 6 | 3.77 | 60.58 |
| | 1' | 3.57 | 62.14 |
| Fructose moiety | 2' | -- | 104.11 |
| | 3' | 4.43 | 77.09 |
| | 4' | 4.34 | 74.37 |
| | 5' | 3.78 | 82.62 |
| | 6' | 3.77 | 62.24 |

and 13.96 for C-1 to C-3 and ω3 to ω1 carbon atoms, respectively and olefinic carbons (C-10, C–11, C–13 and C–14) at (131.61, 128.67, 128.67 and 31.71), respectively[21]. The chemical shift of two allylic carbons (C-9 and C-15 at 28.74 and 28.71), respectively and the bis-allylic carbon (C-12 at 24.49 ppm) suggests that the olefinic protons were cis[22]. Thus, the structure of the compound WB3 was elucidated as 10(cis), 13(cis)-octadecadienoic acid.

*Biological Activities*

*Experimental methods*

In vitro and in vivo biological studies were conducted to determine some pharmacological activities of Bran total extract, WB1 and WB3.

*In vitro study*

*DPPH radical scavenging activity of a whole extract*

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined[32]. A 0.1 ml plant extract in different concentrations (100mg/ml to 8mg/ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard (Ascorbic acid).

*In Vivo studies*

*Acute toxicity study*

The extract was dissolved in distilled water then given orally in graded doses to mice (1, 2, 3, 4 and 5g/kg) with the control group received the same volume of the vehicle. The mortality percentage was recorded three days later. No mortality was reported after three days and according to this; in the typical protocol for acute toxicity study, if this dose levels at 5g/kg (not lethal) it no longer requires for determination of LD50 value[23]. The experimental doses used in the present study was 1/20, 1/10 and 1/5 of (5g/kg) of the Wheat Bran extract (250 and 500 mg/kg).

*Cerebral ischemia induction*

Rats were divided into 5 groups: group 1 for WB1(200 µg/kg), group 2 for WB3(100 µg/kg), group 3 for low dose of whole extract (250mg/kg), group 4 for high dose of whole extract (500mg/kg), group 5 for left common carotid artery occlusion (CCA) group finally, group6 for sham-operated rats. Animals were starved for 12 hours before surgery and after one hour ischemia the test drugs were administered intra peritoneal. All animals were anesthetized with thiopental (50mg/kg)[24]. A longitudinal cervical incision (2cm) was made lateral to the midline and the common carotid artery (CCA) was carefully dissected. Ischemia was induced by placing non traumatic micro vascular clip on the left CCA just prior to its bifurcation[25]. During ischemia rats were monitored for body temperature which was constant at 36.5±0.5˚C using heating pad and respiration pattern. The vascular occlusion was maintained for 30 minutes, and then the clips were removed to resume blood flow to the ischemic region for 24 hours[26]. Finally, the incisions were sutured, the animal was allowed to recover from anesthesia, and returned to a warm cage for recuperation during reperfusion period.

*Biochemistry*

At the end of experimental period, the rats were sacrificed. Brains were rapidly removed, then 0.5g of affected hemisphere was homogenized, the homogenate was centrifuged; the supernatant was taken for the determination of brain level of malondialdehyde (MDA), estimation level of the nitric oxide (NO) metabolites, brain homogenate

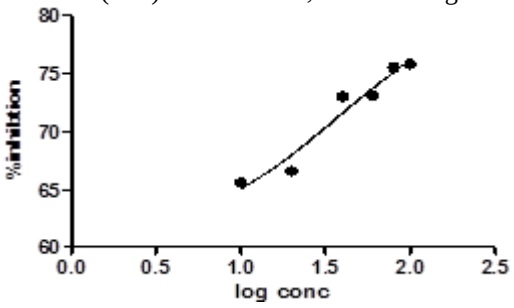



Figure 1: Determination of EC50 of the extract.

Table 3: $^{1}H$ &$^{13}C$ NMR for compound WB3

| No of atoms | $\delta_H$ | $\delta_C$ | No of atoms | $\delta_H$ | $\delta_C$ |
|---|---|---|---|---|---|
| 1 | | 174.51 | 12 | 2.49,t | 24.49 |
| 12 | 1.62, m | 33.66 | 13 | 5.31-5.32 | 128.67 |
| 13 | 2.17, m | 23.24 | 14 | 5.31-5.32 | 131.71 |
| 14 | 1.47 | 29.03 | 15 | 1.97 | 28.71 |
| 15 | 1.97 | 28.74 | 16 (ω3) | 1.33 | 31.29 |
| 16 (ω3) | 5.31-5.32 | 131.61 | 17(ω2) | 1.34 | 22.09 |
| 17(ω2) | 5.31-5.32 | 128.67 | 18( ω1) | 0.84 | 13.96 |

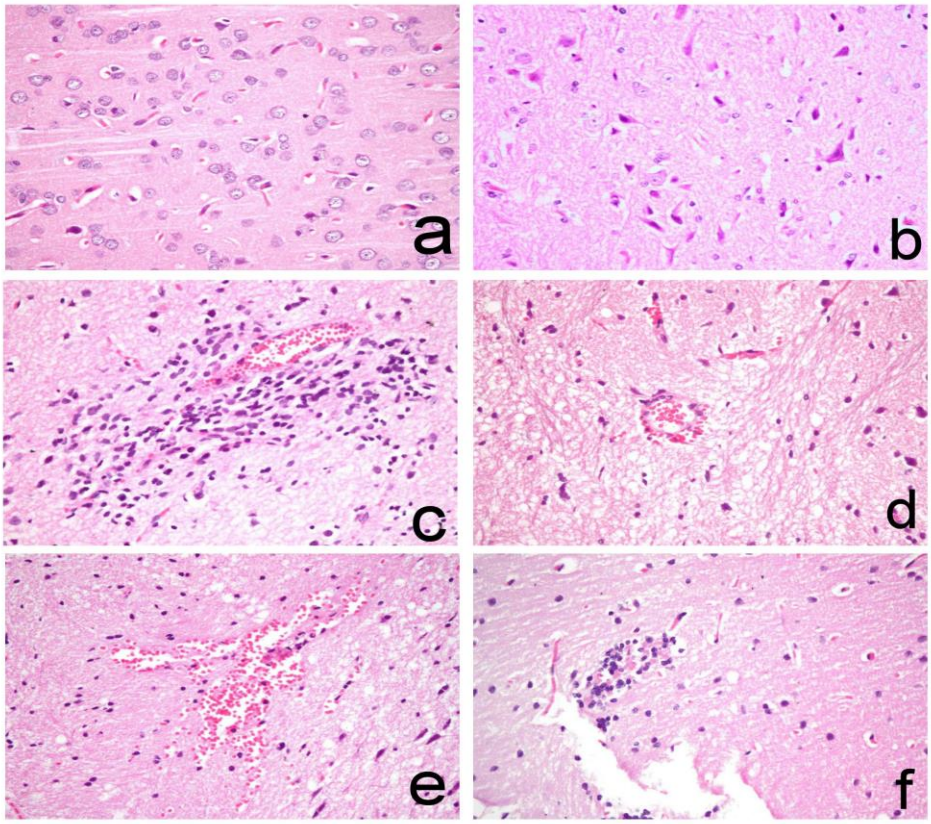



Figure 2: brain of (a)control rat showing normal neuronal cells; ischemic rat showing (b)neuronal cell necrosis, (c) congestion of blood vessels with intense perivascular aggregation of lymphomonocytes and microglia cells, (d) perivascular hemorrhage, (e)focal cerebral hemorrhage and (f) focal area of cerebral tissue necrosis infiltrated by macrophages and glial cells (H&E, X40).

Table 4: Effect of Total extract toward 1, 1-diphenyl-2-picrilhydrazyl (DPPH)

| Extract concentration | % inhibition |
|---|---|
| 10 mg/ml | 65.6 |
| 20 | 66.6 |
| 40 | 73.0 |
| 60 | 73.1 |
| 80 | 75.5 |
| 100 | 75.8 |
| Ascorbic | 81.9 |

(GSH) level and the activities of superoxide dismutase (SOD)[27-30].

*Histopathological examination*

Brain tissues from different groups were fixed in 10% neutral buffered formalin and embedded in paraffin wax. 5µm thick sections were stained with Hematoxylin and Eosin (H&E) and examined using binocular Olympus CX31 microscope. Neuronal cell degeneration and/or necrosis were counted in five cerebral cortical high microscopic fields(x40) and the obtained data were statistically analyzed.

*Immunohistochemical analysis*

Detection of Cyclooxygenase-2 (COX 2) enzyme expression on brain's paraffin sections of control and treated rats using avidin-biotin Peroxidase (DAB, Sigma Chemical Co.) was performed[31]. Tissue sections were incubated with a human monoclonal anti-COX-2 (Cayman Chemical, Ann Arbor, MI, USA). Diaminobenzidene (DAB) was used as a chromogen (DAB, Sigma Chemical Co.) to visualize the immunoreactions. The positive COX-2 immunostained cells were counted in three random cerebral cortical high microscopic fields X40. The obtained results were

Table 5: MDA, NO, GSH content and SOD activity of rat brain

| Group name | MDA nmol / gm tissue | NO nmol/gm tissue | GSH ηmol/gm tissue | SOD U/g tissue |
|---|---|---|---|---|
| Sham | 8.8±0.22 | 14.1±0.45 | 44.4±1.16 | 56.9±3.62 |
| Ischemic | 23.1±1.09∞ | 34.5±2.36∞ | 22.8±1.36∞ | 24.2±0.86∞ |
| WB1 | 10.6±0.87*∞ | 19.9±0.65*∞ | 42.1±1.21* | 53.4±3.83*∞ |
| WB3 | 19.5±0.90∞ | 23.7±1.42*∞ | 30.2±1.07*∞ | 38.7±1.77* |
| Whole extract 250mg/kg | 14.7±1.06*∞ | 22.4±1.53*∞ | 40.9±1.24* | 43.6±2.98*∞ |
| Whole extract 50mg/kg | 19.0±0.92*∞ | 31.1±0.60∞ | 38.2±1.10*∞ | 40.6±1.41*∞ |

Data represent the mean value ±S.E. of six rats per group. Statistical analysis was done using one way ANOVA followed by Turkey for multiple comparisons respectively.
* Significant different from ischemic group at $P < 0.05$.
∞ Significant different from Sham group at $P < 0.05$.

Table 6. Histopathological and immunohistochemical findings

| Groups | Parameters | |
|---|---|---|
| | Neuronal degeneration(cell/ histological field) | Cox2 (cell / histological field) |
| Sham | 0.3±0.33 | 2.3±0.66 |
| Ischemic | 8.0 ±2.30*∞ | 30.3±4.06∞ |
| WB1 | 1.6±0.84*∞ | 8.3±3.76*∞ |
| WB3 | 1.3±0.33*∞ | 9.0±3.79*∞ |
| Whole extract 250mg/kg | 3.6±1.66*∞ | 10.0±2.08*∞ |
| Whole extract 500 mg/kg | 5.3±2.60∞ | 18.0±7.55∞ |

sham, control rats; ischemic, rats of common carotid artery occlusion (CCAO) group; WB1, rats treated with compound WB1(200µg / kg); WB3, rats treated with compound WB3 (100µg / kg); Whole extract 250mg/kg, rats treated with low dose of Whole extract (250mg/kg); Whole extract 500 mg/kg, rats treated with high dose of Whole extract (500mg/kg); Cox2, cyclooxygenase- 2 expression in brain sections counted in three random cerebral cortical high microscopic field per group.
Statistical analysis was done using one way ANOVA.
* Significant different from ischemic group at $P < 0.05$.
∞ Significant different from Sham group at $P < 0.05$.

statistically analyzed using SPSS 17 software.

*Statistical Analysis*

The obtained data was statistically analyzed using ANOVA with Tukey's post-hoc analysis and expressed as mean ± S.E.

**RESULTS**

*In vitro study*

*Antioxidant activity of 85% ethanol extract of Wheat Bran (DPPH radical scavenging activity)*

DPPH, hydrogen acceptor, was used for measuring hydrogen-donating activity of total extract, EC50 value of extract was 38mg/ml fig. (1). the scavenging activity of extract (75.8%) was less than that of ascorbic acid (81.9%) table (4).

*In vivo studies*

*Changes in MDA and NO contents*

There were significantly increased levels of MDA and NO contents in brain tissue of rats subjected to ischemia reperfusion (23.1±1.09nmol/g tissue and 34.5±2.36µmol/g tissue respectively) compared to sham operated rats (8.8±0.22 nmol/g tissue and 14.1±0.45µmol/g tissue, $P< 0.05$). While IP treatment with WB1, WB3 and whole Bran extract (250 and 500mg/kg) prior to ischemia reperfusion showed significant reduction in both MDA and NO levels in the brain tissue compared to the levels measured in ischemic group (Table 5).

*Changes in GSH level and SOD activity*

GSH level and SOD activity in the brain tissue, were significantly decreased in rats subjected to ischemia reperfusion (22.8±1.36 ηmol/gm tissue and 24.2±0.86 U/g tissue respectively) compared to the levels measured in the sham operated control group (44.4±1.16 ηmol/gm tissue and 56.9±3.62 U/g tissue respectively $P< 0.05$), while IP treatment with WB1, WB3 and whole extract (250 and 500 mg/kg) prior to ischemia reperfusion showed significant increased in GSH level and SOD activity in the brain tissue compared to the levels measured in ischemic group (Table 5).

*Histopathology*

The brain of sham operated rats showed normal neurons of cerebral cortex that appeared rounded with large round nuclei and prominent nucleoli (fig.2a) whereas the brain of ischemic groups revealed selective neuronal cell necrosis particularly in cerebral cortex and thalamus. The necrotic neurons appeared angular, shrunken with intensely eosinophilic cytoplasm and pyknotic nuclei (fig.2b) associated with perineuronal and perivascular edema. The degenerated and/or necrotic neuronal cells were significantly increased in ischemic group (8.0 ±2.30 Cell/ Histological field) (table 5) compared to the sham operated one (0.3±0.33 Cell/ Histological fields) (table 6).

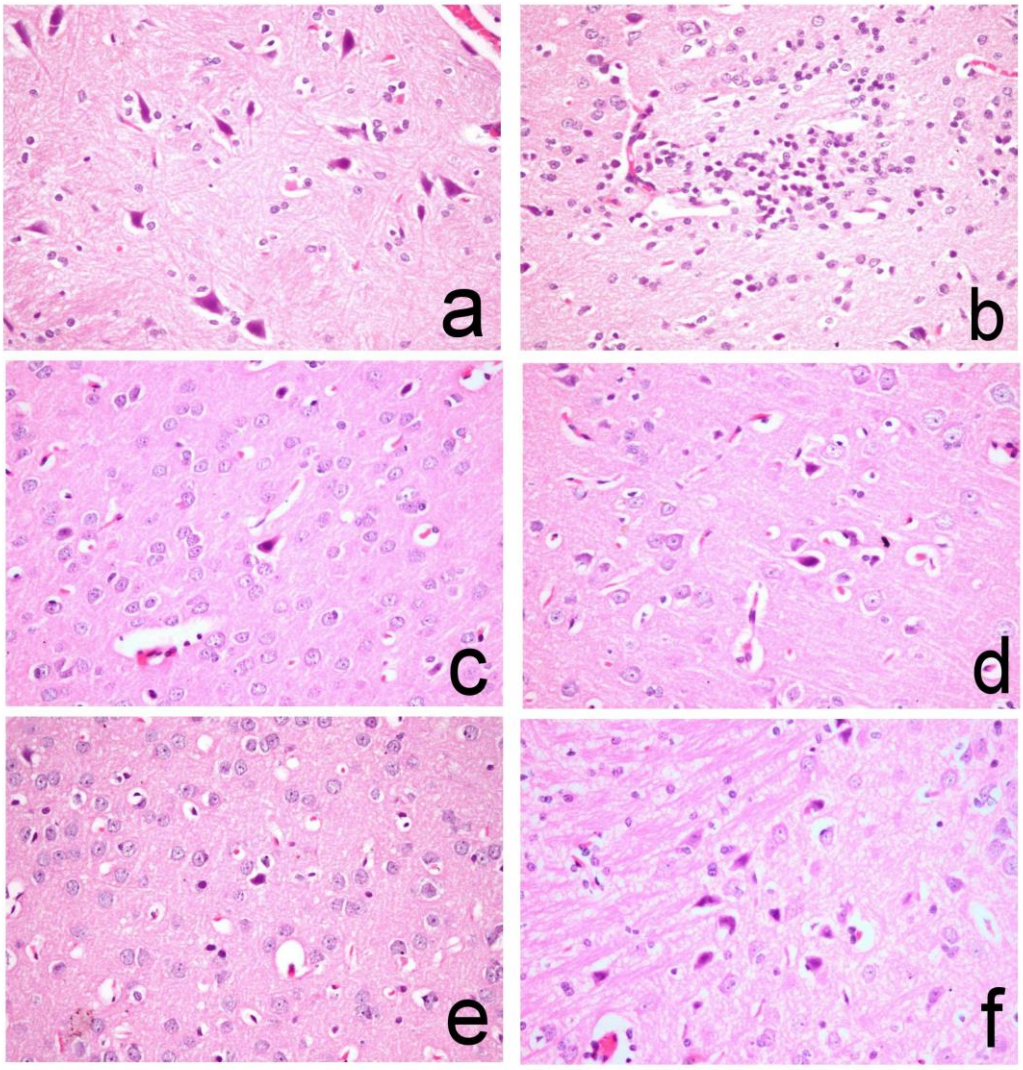



Figure 3: brain of, ischemic rats showing (a) ghost of eosinophilic necrotic neurons associated with neuronophagia (b) gliosis; WB1 treated rat (c) showing sparse necrotic neuronal cells; WB3 treated rat (d) showing decreased number of necrotic cells; whole extract (250mg /kg) (e) showing degenerated neurons and (f) whole extract (500mg /kg) showing eosinophilic neuronal cells. (H&E, X40).

Other lesions were frequently demonstrated as congestion of blood vessels with intense perivascular aggregation of lymphomonocytes and microglia cells (fig.2c) and perivascular as well as focal cerebral hemorrhage (fig.2d & 2e). In addition, lesions in focal area of cerebral tissue were demonstrated as tissue necrosis infiltrated by macrophages and glial cells (fig.2f). Neurons of thalamic area revealed ghost of eosinophilic necrotic neurons associated with neuronophagia (fig.3a). The blood capillaries in the ischemic area revealed endothelial hypertrophy associated with diffuse gliosis with presence of active rod shape microglia cells (fig.3b). These histopathological alterations were regressed in other pretreated groups with significant decreased number of necrotic cells in WB1 and WB3 treated groups (fig. 3c&3d) (1.6±0.84 and1.3±0.33 Cell/ Histological field respectively) (table 6) compared to ischemic one. Sparse necrotic neuronal cells were demonstrated in whole extract (250 and 500 mg/kg) treated groups (fig. 3e and 3f) with no significant difference between them (3.6±1.66 and 5.3±2.60 Cell/ Histological field respectively) (table 6).

*Immunohistochemistry*

Brain of sham operated control rats showed sparse COX-2 positive cells (fig. 4a) (2.3±0.66 Cell/Histological field) (table 6). Meanwhile, brain of ischemic group showed abundant COX-2 positive neurons (30.3±4.06 Cell/Histological field) (table 6) with perinuclear immune reactivity (fig. 4b). The number of COX-2 immunereactive cells were significantly decreased in WB1, WB3 and whole extract (250 mg/kg) treated groups (fig.4c, 4d &4e) (8.3±3.76, 9.0±3.79 and 10.0±2.08 Cell/Histological field respectively) (table 6), compared to the ischemic one with no significant difference between them and whole extract (500 mg/kg) treated group showed no significant difference (fig.4f) (18.0±7.55 Cell/Histological field) (table 6) compared to the ischemic one.

**DISCUSSION**

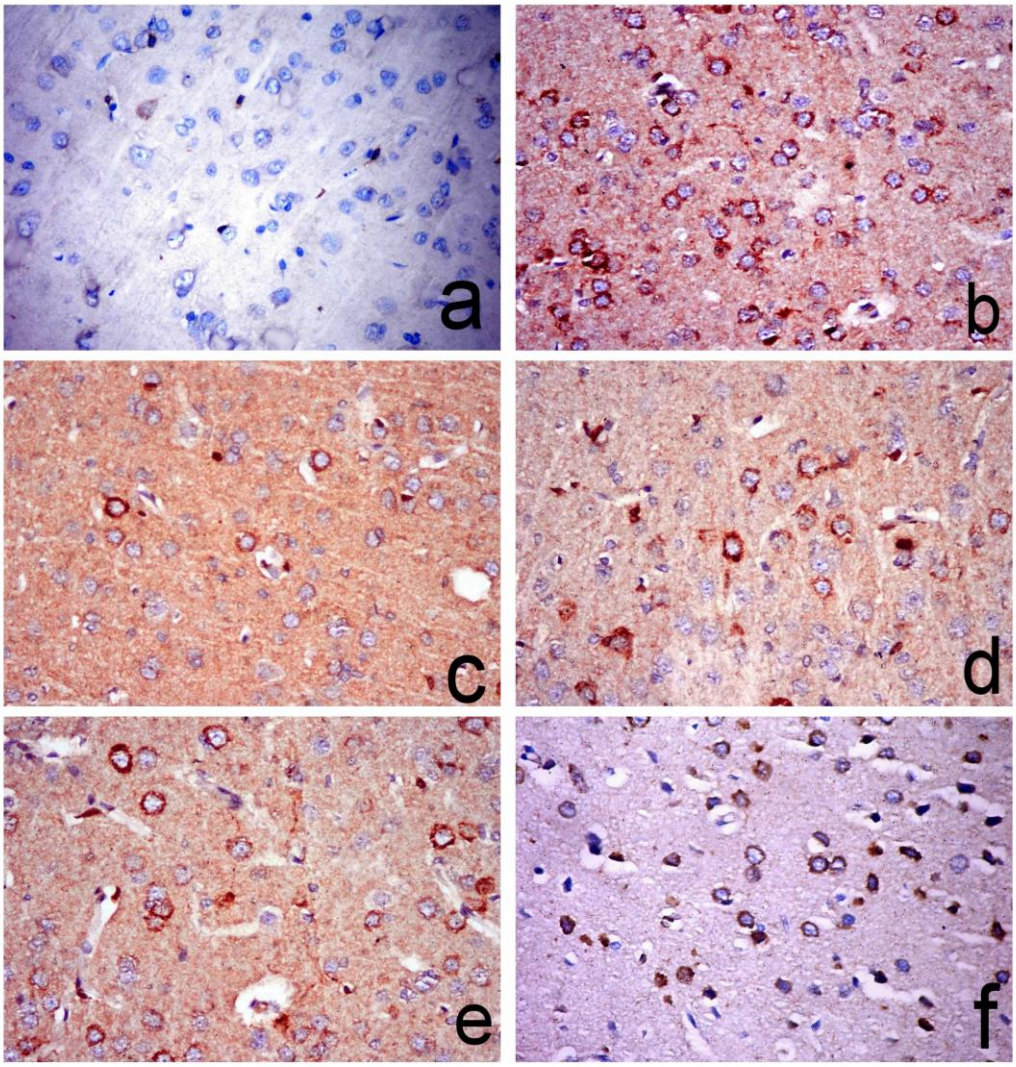



Figure 4: Immunohistochemical staining of brain of, control rat (a) showing sparse COX-2 positive cells; ischemic rat (b) showing abundant COX-2 positive neurons; WB1 treated group (c) showing significantly decreased COX-2 positive neurons; WB3 treated group (d) showing COX-2 positive neurons; whole extract (250mg/kg) treated group (e) showing COX-2 positive neurons and whole extract (500mg/kg) (f) treated group showing abundant COX-2 positive neurons (H&E, X40).

According to this study, rats subjected to cerebral ischemia for 30 min then followed by reperfusion for 24 hours had significantly higher increase in the brain tissue levels of MDA and NO and the decrease in GSH content and SOD activity compared to sham-operated rats. The reactive oxygen species (ROS) during ischemia-reperfusion (I/R) has a direct role for the level of brain injury[33,34]. Furthermore, accumulation of ROS through I/R increases the incidence of apoptotic cell death in the brain[35]. In a word, WBI, WB3 and total ethanol Bran extract 250mg/kg ameliorated the oxidative stress resulted from cerebral ischemia-reperfusion. The mechanisms of the neuroprotective effects of the extract on cerebral ischemia-reperfusion injury are not fully clear, but the effects can be referred to its anti-oxidant as it can scavenge a number of reactive species. Moreover, it restored the I/R-induced depletion of the activity of SOD and GSH contents, while reduced the amount of MDA and NO contents that elevated during cerebral ischemia-reperfusion injury. Pronounced histopathological alterations represented by eosinophilic neuronal cell necrosis, ghost neurons, gliosis with neuronophagia, cerebral hemorrhage and spongiosis were demonstrated in the ischemic group. Similar results were recorded, these alterations denoting ischemia induced by ligation of internal carotid artery with reduction in cerebral blood flow and deprivation of oxygen and glucose delivery that induce inflammation and oxidative stress which lead to neuronal death[36]. Neuronal death could occur via three major mechanisms, including apoptosis, autophagia and coagulative necrosis, in response to an ischemic insult[37]. Neurons are the most vulnerable to brain ischemia, because of the high content of neuronal membrane with polyunsaturated fatty acids that is considered a target of free radical which induce lipid peroxidation of neuronal cell membrane with release

of fatty acid hydro peroxides which are able to accentuate free radical reactions and increasing the damage[38]. The role of mitochondria in ischemic brain injury which include decrease ATP synthesis, induction of free radical production and formation of mitochondrial pore with release of Cytochrome C that enhance apoptotic cell death[39]. These histopathological lesions were ameliorated in group treated by β-sitosterol because of its neuroprotective effect in neurodegenerative disorder[40]. More over β-sitosterol has anti inflammatory effect through inhibition of inflammatory mediators particularly Tumor necrosis factor alpha[41]. Recently they confirmed that β-sitosterol has antioxidant activity through stimulation of antioxidant enzyme by activation of estrogen receptor / P13- kinase – dependant pathway and scavenging ROS. Meanwhile β-sitosterol was not able to inhibit the Cyclooxigenase (COX) pathway[42]. Concerning the immunohistochemical result, the ischemic group showed increased numbers of positively immune reactive cells. Several studies have correlated the Cyclooxygenase 2 (COX-2) expression and ischemic neuronal death[43]. These studies have confirmed the expression of COX-2 in vulnerable neurons after global and focal. Neuronal over expression of COX-2 accelerate neuronal apoptosis, participate in inflammation-mediated cytotoxicity and consequently accelerate cerebral infarction[44].

## CONCLUSION

The present study showed that wheat bran extract and its active constituents have neuroprotective effect through their antioxidant activity.

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