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Full Length Research Paper

Simultaneous spectrophotometric determination of phenacetin and paracetamol in human plasma for the quantitative assessment of liver function reserve

Peng Yang¹, Lei Shi¹, Lin-Na Liu¹, Xin-You Liu¹, Xiang-Rui Wang², Ping He² and Yan Zhang^{1*}¹Department of Pharmacy, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China.²Department of Anesthesiology, Renji Hospital Affiliated to School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China

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A simple, economical and reliable spectrophotometric method was developed for the simultaneous determination of phenacetin and paracetamol in human plasma. We purified the plasma samples by solid phase extraction procedure. The analytes reacted with chromogenic sodium 1,2-naphthoquinone-4-sulfonate and cetyltrimethyl ammonium bromide, and the products conformed to Beer's law over the range 2 to 24 µg/ml for phenacetin and 1 to 16 µg/ml for paracetamol. The method was validated according to the Food and Drug Administration (FDA) guideline. There was no statistical difference between the proposed method and the modified high performance liquid chromatography (HPLC) method with regard to accuracy and precision. The proposed method is suitable for the quantitative assessment of liver function reserve in patients with liver cirrhosis.

Key words: Spectrophotometric method, phenacetin, paracetamol, liver function reserve.

INTRODUCTION

The assessment of liver function reserve is critically important for the prediction of the safety of partial hepatectomy and the efficacy of a therapeutic intervention in patients with liver cirrhosis. Phenacetin test is a useful method for the quantitative assessment of liver function reserve (Schneider, 2004; Qu et al., 2007; Liu et al., 2012). The test is to measure the ratio of plasma concentration of the metabolite-paracetamol (PAR) to the probe drug-phenacetin (PHN) at a time after oral administration of phenacetin (Xiong et al., 2010). Hence, it is essential to develop a simple, economical and reliable method for simultaneous determination of PAR and PHN in plasma.

A few methods have been reported for simultaneous

determination of PHN and PAR in plasma including high performance liquid chromatography (HPLC) (Gotelli et al., 1977; Uges et al., 1981; Mineshita et al., 1989; Bartoli et al., 1996; Cui et al., 2002), liquid chromatography-mass spectrometry (LC-MS/MS) (Xiong et al., 2010; Whiterock et al., 2012) or gas chromatography-mass spectrometry (GC-MS) (Murray et al., 1991). It is too complicated and costly of each of these methods to be applied in routine clinical work.

Although some simple spectrophotometric methods have been reported for determination of PHN or PAR in pharmaceutical preparation (Michael et al., 1989; Domagalina et al., 1997; Nagaraja et al., 1998; Yousefinejad and Hemmateenejad, 2012), there is not

*Corresponding author. E-mail: ypill@163.com. Tel: 86-29-84777154. Fax: 86-29-84777154.

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any spectrophotometric method reported for simultaneous determination of PHN and PAR in complicated samples such as plasma. In this paper, a simple and economical spectrophotometric method was developed for simultaneous determination of PHN and PAR in plasma and validated according to the United States Food and Drug Administration (US FDA's) Guidance for Industry Bioanalytical Method Validation.

The PHN and PAR in the plasma samples were purified by solid phase extraction (SPE) and reacted with sodium 1,2-naphthoquinone-4-sulphonate (NQS) and cetyltrimethyl ammonium bromide (CTAB) to increase the absorptivity. The concentrations of PHN and PAR were determined by the simultaneous equation method. Analytical accuracy and precision of the proposed method were compared statistically with a modified HPLC method considering its high selectivity and sensitivity. The proposed method was successfully applied in the phenacetin test to quantitatively assess liver function reserve of eight healthy subjects and twenty-two cirrhotic patients. The proposed method is suitable for the phenacetin test to be used in routine clinical work.

MATERIALS AND METHODS

Chemicals and reagents

Pure phenacetin, paracetamol and acetanilide were purchased from Sigma-Aldrich (Prague, Czech Republic). Sodium 1,2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium bromide were purchased from Aladdin (China). All other reagents were of analytical grade.

Apparatus

A SHIMADZU model OV-265 double beam UV-Vis spectrophotometer with a fix slit width of 1 nm coupled 1 cm quartz cell was used for spectral measurements. The chromatographic system consisted of an Agilent 1200 series LC system equipped with an G1322A solvent degasser, G1311A pump, G1314B detector, G1329A autosampler, Agilent ChemStation B.04.02 and a reversed phase 5 μ m Xterra ODS column (250 \times 4.6 mm, i.d.). All pH measurements were made with a SARTORIUS model PB-10 digital pH meter. Empty SPE tubes and frits for Solid-phase extraction (SHENZHENBIOCAMMA BIOTECH CO., LTD), and AMBERCHROM™ CG161M macroreticular adsorbent (Rohm and Haas) were used.

Solution preparation

Preparation of calibration standards

A 10 mg/ml stock solution of PAR was prepared by dissolving accurately weighted pure PAR in ethanol. It was serially diluted to give working solutions at the concentrations over 0.1 to 1.6 mg/ml, after that the calibration standards of PAR at the concentrations of 1, 2, 4, 8, 12, 16 μ g/ml were prepared by spiking 2 ml of blank human plasma with 20 μ l of working solutions. Calibration standards of PHN at the concentrations of 2, 4, 8, 12, 16, 24 μ g/ml were prepared using the same method.

Preparation of quality control (QC) samples

The QC samples were prepared by spiking blank human plasma at concentrations of 2, 10, 24 μ g/ml for PHN and 1, 10, 16 μ g/ml for PAR, which represented low, medium, and high concentration QC samples, respectively. The QC samples were prepared independently of the calibration standards.

Preparation of reaction reagent solutions

A 0.1% (M/V) aqueous solution of NQS was freshly prepared and protected from sunlight. A 1% (M/V) aqueous solution of CTAB, 40% (M/V) sodium hydroxide solution (NaOH) and 0.4 % (M/V) sodium hydroxide solution (NaOH) were used.

Sample preparation and determination for spectrophotometric method

An aliquot of 5 ml of calibration standard, QC sample or unknown plasma sample was loaded onto the CG161 cartridge that was preconditioned with 2 ml of 95% ethanol and 9 ml of water in order. It was then washed with 6 ml of deionized water. The elution was made with 2 ml of 65% ethanol. The elution collected was mixed with 0.8 ml of 4 M HCl, and refluxed for 1 h. The solution was cooled to room temperature and transferred to a 5 ml calibrated flask, and 1.16 ml of 40% NaOH, 0.25 ml of 0.4% NaOH and 200 μ l of 0.1% NQS were added sequentially. The mixture was vortexed, left to stand for ten minutes, and 200 μ l of 1% CTAB was then added. The mixture was filled to the mark with deionized water. After mixing the solution thoroughly, an aliquot of this analyte was taken and the absorbance was measured at 500 and 565 nm against the reagent blank according to the standard procedure. Absorbance value (A) was substituted into the corresponding equation to get the plasma concentrations (C).

Chromatographic conditions

The chromatographic conditions of the modified HPLC method were as follows: Plasma sample of PHN and PAR was mixed with acetanilide as an internal standard and purified by the SPE procedure shown earlier; an aliquot of 20 μ l of the elution was injected into the HPLC system and analysed at 245 nm; the mobile phase was water and methanol (63.5:36.5, v/v), which run at a flow rate of 1 ml/min at 37°C.

RESULTS AND DISCUSSION

Development of a solid-phase extraction procedure (SPE)

The previous purification methods reported for the determination of PAR and PHN in plasma included methanol deproteinization (Uges et al., 1981) and more complex liquid-liquid extraction (LLE) using acetoacetate (George et al., 1977). Both have obvious disadvantages. Too much organic solvent is added into the plasma in protein precipitation. Besides that, LLE has a difficulty in the stable reproducibility of the extraction due to the

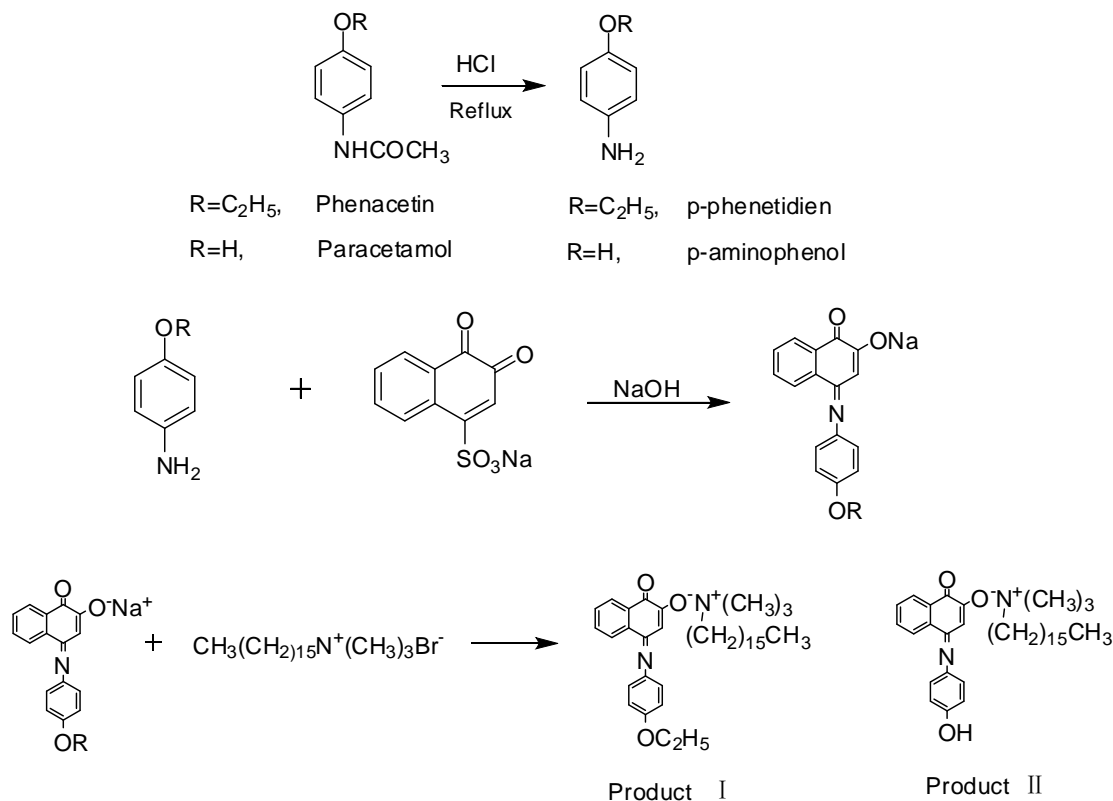


Figure 1. The reaction mechanism.

incomplete phase separation. A solid-phase extraction (SPE) was developed for the sample preparation of PHN and PAR in plasma. Different kinds of nonpolar sorbents were tested in this research including polystyrene (PS), octyl (C8) and octadecyl (C18). The best purification and satisfied recovery values for PHN and PAR obtained were using polystyrene column (AMBERCHROM™ CG161M macroreticular adsorbent). The absolute extraction recoveries for low, medium and high concentration QC samples were consistent at 98% using 0.165 g CG161M with 65% ethanol as elution at room temperature. The absolute extraction recoveries of PAR and PHN from the SPE procedure were much higher than the average 80% of the LLE reported in literature (George et al., 1977), and no organic solvent was involved in the SPE procedure.

Reaction mechanism

Because the analysing sensitivity of spectrophotometric method is generally lower than HPLC, LC-MS/MS and GC-MS, NQS and CTAB have been added as the chemical derivative chromogenic reagents to increase the analysing sensitivity of the proposed method. PHN reacted with NQS and CTAB to form product I that had higher absorptivity than PHN itself, and PAR was handled

in the same way to form product II. The stoichiometric ratio of reagent and corresponding product in this reaction was 1:1. The reaction mechanism is shown in Figure 1.

Influence of pH

The effects of reaction pH on the absorbance of products were studied, and pH 8 to 9 was selected as the optimal experimental conditions. When pH was above nine, the absorbance of the solution could hardly stabilize but increased slowly. A previous paper (Nagaraja et al., 1998) reported that, PAR solution was stable with NaOH, but PHN solution was not. Na_2CO_3 was used instead of NaOH in their paper. However, we found that the stability of absorbance was only influenced by the pH of the solution, not influenced by the chosen of pH regulator. In this study, we chose NaOH as the pH regulator in the reaction.

Influences of temperature

Keeping pH at nine, the influence of temperature on the reaction was studied. We found that the absorbance of solution was maximal at room temperature and decreased

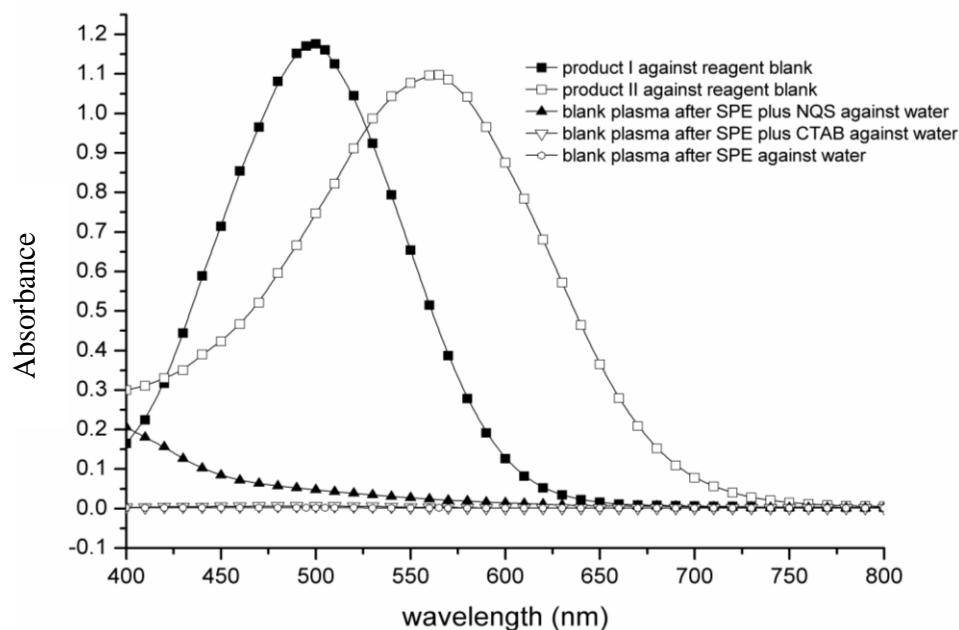


Figure 2. Absorption spectra of I, II, NQS, CTAB, and blank plasma.

decreased with the increased temperature. The absorbance of the solution fell to zero in water bath at 80°C for 10 min. Therefore, we chose room temperature as the optimum reaction condition in order to make the method reproducible and simple.

Influence of reaction time

Keeping other conditions unchanged, the absorbance of the product was measured at different reaction time. The results showed that both p-aminophenol and p-phenetidine reacted quickly with NQS and CTAB. The absorbance increased to the top within 5 min and kept stable in 24 h. Therefore, no less than 10 min was necessary for the reaction to complete considering the robustness.

Spectral characteristics

The spectra of the product I and product II were determined separately. Overlain spectra (Figure 2) suggested that the product I and product II showed maximum absorbance at 500 and 565 nm, respectively. The solutions were found to be sufficiently stable throughout the experiment.

The establishment of simultaneous regression equations

Both the product I formed from PHN and product II formed

formed from PAR interfered with the absorption of each other. However, Figure 2 showed different absorption maxima. Therefore, modified Beer's law (Chaudhari et al., 2006) (law of additivity) was used for their estimation in mixture (Dave et al., 2007; Garg et al., 2008).

$$A_{500} = 0.0325C_{PHN} + 0.0282C_{PAR} \quad (1)$$

$$A_{565} = 0.0126C_{PHN} + 0.0594C_{PAR} \quad (2)$$

Where, C_{PHN} and C_{PAR} were the concentrations of PHN and PAR in the plasma, A_{500} and A_{565} were absorbance values of the sample solution at 500 nm and 565 nm, respectively.

Values of 0.0325 and 0.0126 were the mean apparent absorptivity of PHN at 500 nm and 565 nm, respectively. Values of 0.0282 and 0.0594 were the mean apparent absorptivity of PAR at 500 nm and 565 nm, respectively. The mean apparent absorptivity of PHN and PAR were determined by measuring the absorbance of different concentrations of calibration standards at the selected wavelengths (Table 1).

By applying the Cramer's rule to Eqn. 1 and 2, the C_{PHN} and C_{PAR} can be obtained as follows:

$$C_{PHN} = \frac{0.0594A_{500} - 0.0282A_{565}}{0.00157} \quad (3)$$

$$C_{PAR} = \frac{0.0325A_{565} - 0.0126A_{500}}{0.00157} \quad (4)$$

So, the concentrations of PHN and PAR in unknown samples can be calculated by Eqn. 3 and 4, respectively.

Table 1. Absorptivity values of PHN and PAR at 500 nm and 565 nm (mean ± SD, n = 6).

Absorptivity at 500nm		Absorptivity at 565nm	
PHN	PAR	PHN	PAR
0.0325 ± 0.0013	0.0282 ± 0.0011	0.0126 ± 0.0005	0.0594 ± 0.0026

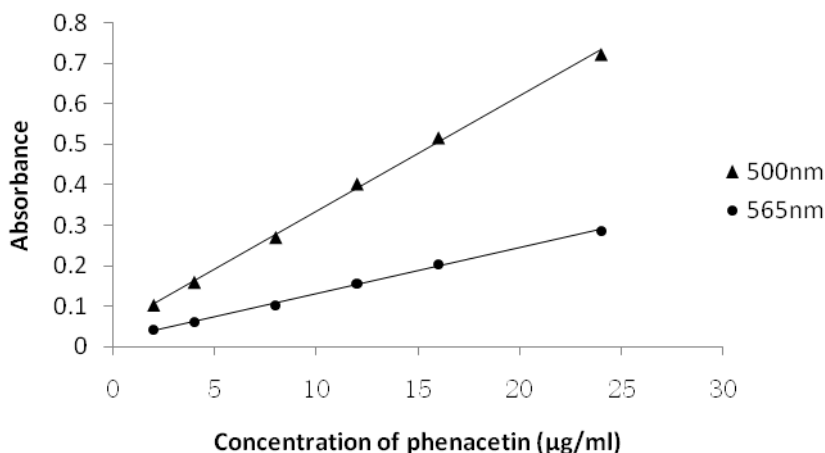


Figure 3. Calibration curve of PHN at 500 and 565 nm.

Method validation

The method was validated according to the US FDA’s Guidance for Industry Bioanalytical Method Validation (2000). The calibration curves were constructed for both PHN and PAR at 500 and 565 nm using the calibration standards (Figures 3 and 4). Beer’s law hold well over the range 2 to 24 µg/ml for PHN and 1 to 16 µg/ml for PAR and the calibration curves covered the entire range of clinical expected concentrations (Qu et al., 2007). The precision of the proposed method was determined at low, medium and high concentrations by analysing six QC samples over 1 day in random order (Intraday precision or within-run precision), and analysing each control once on each of the six different days (Interday precision or intra-batch precision). The sensitivity of the method was measured by determination of the lower limit of detection (LLOD) and lower limit of the quantification (LLOQ). LLOD and LLOQ of PHN and PAR at 500 and 565 nm were calculated, respectively, according to the Eqn. 5 and 6, where σ is the standard deviation of reagent blank and s is the slope of the calibration curve.

$$LLOD = \frac{3.3\sigma}{s} \tag{5}$$

$$LLOQ = \frac{10\sigma}{s} \tag{6}$$

The results are satisfactory and presented in Table 2. The absolute extraction recoveries were the absorbance

values obtained from three concentrations of PHN and PAR added to and extracted from QC plasma samples, compared with the absorbance values obtained for the true concentrations of the pure authentic unextracted standards prepared with 65% ethanol instead of blank human plasma. The absolute extraction recoveries were consistent at 98% of the low, medium and high concentrations indicated the good extraction efficiency of the SPE procedure. Method’s accuracy was evaluated by means of recovery studies of QC samples at three concentration levels (n = five for each level). The results are satisfactory: mean recovery values are always higher than 95%; the deviations of the mean (%Bias) from the true recovery values are within 4% that is much less than the 15% requested in the FDA guideline (Table 3).

Statistical comparison of the results of the proposed method and the HPLC method

Spiked QC samples at concentrations of 2, 12, 24 µg/ml for PHN and 1, 8, 16 µg/ml for PAR were measured by both the proposed method and the HPLC method. The results obtained were presented in Table 4 and compared statistically by the statistical package for social sciences (SPSS) 15.1. Student’s t-test and variance ratio F-test were examined for accuracy and precision at 95% confidence level, respectively. The calculated t-values and F-values at all the three concentrations did not exceed

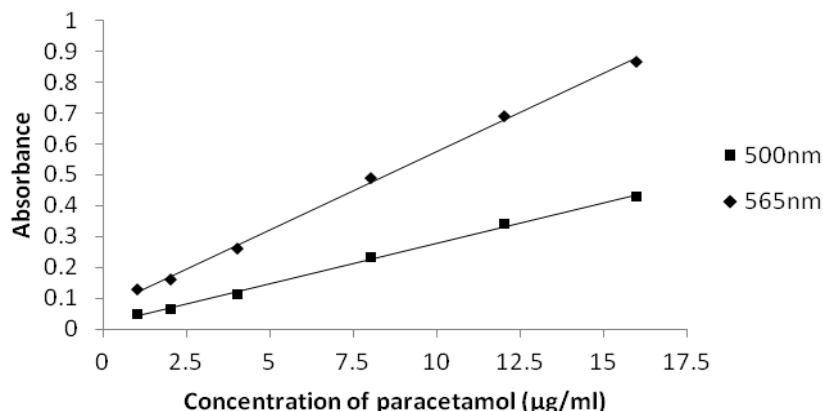


Figure 4. Calibration curve of PAR at 500 and 565 nm.

Table 2. Calibration data of PAR and PHN.

Parameter	PHN		PAR	
	500	565	500	565
Analytical wavelength (nm)	500	565	500	565
Molar absorptivity (l/mol/cm)	5.10×10^3	2.02×10^3	3.97×10^3	7.65×10^3
Linearity range (µg/ml)	2.0–24.0	2.0–24.0	1.0–16.0	1.0–16.0
Regression equation	$0.0285x+0.0491$	$0.0113x+0.0178$	$0.0263x+0.0169$	$0.0506x+0.0703$
Slope	0.0285	0.0113	0.0263	0.0506
S.E. of slope	0.0005	0.0002	0.0007	0.0011
Intercept	0.0491	0.0178	0.0169	0.0703
S.E. of intercept	0.0056	0.0022	0.0027	0.0053
Correlation coefficient (R^2)	0.9984	0.9976	0.9971	0.9981
LLOD (µg/ml)	0.583	0.620	0.312	0.291
LLOQ (µg/ml)	1.766	1.879	0.947	0.882
Intraday precision (%RSD)				
Low concentration	5.2		4.3	
Medium concentration	2.4		2.8	
High concentration	1.2		1.4	
Interday precision (%RSD)				
Low concentration	6.7		5.7	
Medium concentration	2.7		2.4	
High concentration	1.3		1.4	

exceed the theoretical values ($t = 2.776$, $F = 19.00$; $n = 3$) indicating that there was no significant difference between the proposed method and the HPLC method with regard to accuracy and precision. The chromatograms of the pure analyte and QC sample are shown in Figure 5.

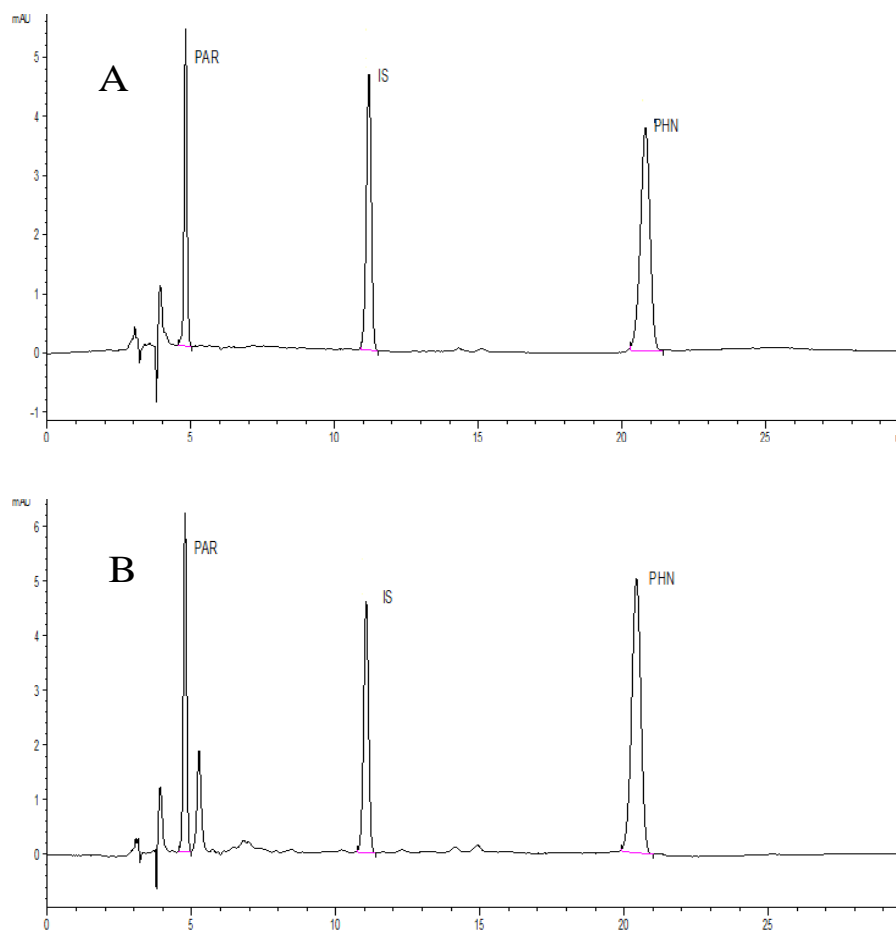
Analytical application in phenacetin test

Eight healthy subjects and twenty-two cirrhotic patients participated in phenacetin test to assess the liver function reserve from November, 2011 to February, 2012. All of

the participants were required to fast overnight. Phenacetin 1 g was ingested with 200 ml water in the morning. The blood sample was drawn from antecubital vein at 2 h after the oral administration of phenacetin. The contents of phenacetin and paracetamol in plasma were determined by both the proposed method and the HPLC method. The results are shown in Table 5. Significant differences in the ratio of plasma concentration of PAR to PHN were observed between the controls and the cirrhotic patients using two-sample t-test at 95% confidence level. The ratio of plasma concentration of PAR to PHN calculated with the proposed method decreased 60.8% in the cirrhotic patients (0.381) compared

Table 3. Accuracy of the proposed method calculated at low, medium and high concentration of the spiked QC plasmas (mean \pm SD, n=5).

Sample NO.	Added ($\mu\text{g/ml}$)		Found ($\mu\text{g/ml}$)		Recovery (%)		%Bias		%RSD	
	PHN	PAR	PHN	PAR	PHN	PAR	PHN	PAR	PHN	PAR
1	2	16	1.94 \pm 0.12	16.28 \pm 0.22	97.0	101.8	-3.0	1.8	6.18	1.35
2	10	10	10.12 \pm 0.23	9.81 \pm 0.16	101.2	98.1	1.2	-1.9	2.27	1.63
3	24	1	24.43 \pm 0.36	1.04 \pm 0.07	101.8	104.0	1.8	4.0	1.47	6.73

**Figure 5.** Representative chromatograms obtained from (A) pure PHN, PAR and I.S. (B) QC sample.

compared with the controls (0.972). There was no statistical difference of the ratio between the proposed method and the HPLC method (60.8 vs. 58.8%) using paired t-test at 95% confidence level. The decreased ratio indicated that the liver function reserve in cirrhotic patients had been much lower. The spectrophotometric method was successfully applied in the phenacetin test of cirrhotic patients and healthy subjects.

Conclusion

A simple and reliable spectrophotometric method for the

simultaneous determination of phenacetin and paracetamol for the quantitative assessment of liver function reserve has been developed and validated. A major improvement of the proposed method is that it can be used for analysis in human plasma compared with the spectrophotometric methods reported before. In contrast with the HPLC, LC-MS and GC-MS methods, the proposed method has many advantages: the extraction recovery is higher; it avoids using organic reagent; the test fee per sample is lower. What is more, spectrophotometers are more popular than HPLC, LC-MS or GC-MS equipped in hospitals, and spectrophotometric method

Table 4. Comparison of QC samples determined by the proposed method and the HPLC method.

Sample		Concentrations analysed (mean \pm SD, n=3)		Statistic	
		Proposed method	HPLC method	$F_{\text{calculated}}$	$t_{\text{calculated}}$
Low	PHN	2.04 \pm 0.11	2.02 \pm 0.06	3.36	0.276
	PAR	0.96 \pm 0.06	0.98 \pm 0.02	9.00	0.5 48
Medium	PHN	12.12 \pm 0.26	12.06 \pm 0.13	4.00	0.358
	PAR	8.03 \pm 0.17	8.01 \pm 0.11	2.39	0.171
High	PHN	23.73 \pm 0.32	24.03 \pm 0.20	2.56	1.377
	PAR	16.28 \pm 0.21	16.12 \pm 0.14	1.25	1.098

Table 5. Determination of paracetamol and phenacetin in phenacetin test compared with the HPLC method (mean \pm SD).

Sample	PHN ($\mu\text{g/ml}$)	PAR ($\mu\text{g/ml}$)	Ratio	PHN ($\mu\text{g/ml}$, HPLC)	PAR ($\mu\text{g/ml}$, HPLC)	Ratio (HPLC)
Controls	4.68 \pm 2.69	4.55 \pm 2.64	0.972 Δ	4.74 \pm 2.72	4.52 \pm 2.23	0.954
Cirrhotic patients	7.06 \pm 3.03	2.69 \pm 1.88	0.381* Δ	7.03 \pm 2.63	2.76 \pm 1.69	0.393*

* $P < 0.05$ vs. controls; $\Delta P > 0.05$ vs. HPLC method.

may save more time to analyse batches of samples. Hence, the proposed method is more practical and suitable for the phenacetin test to be used in routine clinical work.

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REFERENCES

- Bartoli A, Xiaodong S, Gatti G, Cipolla G, Marchiselli R, Perucca E (1996). The influence of ethnic factors and gender on CYP1A2-mediated drug disposition: a comparative study in Caucasian and Chinese subjects using phenacetin as a marker substrate. *Ther. Drug Monit.* 18(5):586-591.
- Cui ZF, He P, Luo M, Xia SQ, Wu MC (2002). Phenacetin O-deethylation in extrahepatic tissues of rats. *Eur. J. Drug Metab. Pharmacokinet.* 27(2):107-111.
- Chaudhari SV, Karnik A, Adhikary A, Tandale RS, Vavia PR (2006). Simultaneous UV spectrophotometric method for the estimation of cefuroxime axetil and probenecid from solid dosage forms. *Indian J. Pharm. Sci.* 68(1):59-63.
- Dave HN, Mashru RC, Thakkar AR (2007). Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. *Anal. Chim. Acta.* 597(1):113-120.
- Garg G, Saraf S, Saraf S (2008). Spectrophotometric and column high-performance liquid chromatographic methods for simultaneous estimation of metoprolol tartrate and hydrochlorothiazide in tablets. *J. AOAC. Int.* 91(5):1045-1050.
- Gotelli GR, Kabra PM, Marton LJ (1977). Determination of acetaminophen and phenacetin in plasma by high-pressure liquid chromatography. *Clin. Chem.* 23(6):957-959.
- Liu Z, Qu Z, Li X, Cai M, He P, Zhou M, Xiao J, Wang X (2012). Phenacetin O-Deethylation is a Useful Tool for Evaluation of Hepatic Functional Reserve in Rats with CCl(4)-Induced Chronic Liver Injury. *J. Surg. Res.* 175(2):e61-66.
- Mineshita S, Toyoshima A, Yazaki T (1989). Influence of phenacetin and its metabolites on renal function. *Nihon Jinzo Gakkai Shi.* 31(6):629-633.
- Nagaraja P, Murthy KC, Rangappa KS (1998). Spectrophotometric method for the determination of paracetamol and phenacetin. *J. Pharm. Biomed. Anal.* 17(3):501-506.
- Qu ZQ, Li XD, Liu HL, He P, Zhang X, Wu MC (2007). Impaired clearance of phenacetin in hepatic cirrhosis and fibrosis. *Int. J. Clin. Pharmacol. Ther.* 45(1):55-62.
- Schneider PD (2004). Preoperative assessment of liver function. *Surg. Clin. N. Am.* 84:355-373.
- Uges DRA, Bloemhof H, Juul Christensen EK (1981). An HPLC method for the determination of salicylic acid, phenacetin and paracetamol in serum, with indications; two case-reports of intoxication. *Pharm. World Sci.* 3(1):1312-1315.
- US Food and Drug Administration, Center for Drug Evaluation and Research (CDER) (2000). Guidance for the Industry: Analytical Method Validation. Rockville, Md, USA.
- Whiterock VJ, Morgan DG, Lentz KA, Orcutt TL, Sinz MW (2012). Phenacetin pharmacokinetics in CYP1A2-deficient beagle dogs. *Drug. Metab. Dispos.* 40(2):228-231.
- Xiong WJ, Jin H, Li SJ, Jin JC, Ji BN, Yu C (2010). Evaluating human liver reserve function by measuring serum concentrations of phenacetin and its metabolites. *J. Dig. Dis.* 11(6):358-363.
- Yousefinejad S, Hemmateenejad B (2012). Simultaneous spectrophotometric determination of paracetamol and paracetaminophenol in pharmaceutical dosage forms using two novel multivariate standard addition methods based on net analyte signal and rank annihilation factor analysis. *Drug Test Anal.* 4(6):507-514.

Full Length Research Paper

Resveratrol alleviates oxidative stress and inflammation in the hippocampus of rats subjected to global cerebral ischemia/reperfusion: Comparison with vitamin E

Zainab K. Mahdi*, Rania M. Abdelsalam and Azza M. Agha

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Egypt.

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Resveratrol is a dietary polyphenol present in nuts and dried fruits. It is a potent antioxidant and anti-inflammatory agent; these properties led researchers to investigate its protective effects in several animal models of neurological disease, particularly those in which inflammation and oxidative stress may play a role in their pathogenesis. Thus it seemed interesting to study the possible neuro-protective effect of resveratrol and vitamin E in cerebral ischemia/reperfusion (IR)-induced hippocampal damage in brains of rats. Rats were divided into four groups (n=10); groups 1 and 2 were given 0.5 ml (1% tween 80, po) while groups 3 and 4 were treated with vitamin E (100 mg/kg, po) and resveratrol (10 mg/kg po), respectively. All treatments were administered for 14 days and on the 15th day of the experiment, animals were anaesthetized with thiopental (50 mg/kg, ip) and IR was induced by occlusion of both carotid arteries for 60 min, followed by reperfusion for another 60 min in all groups except for the sham-operated group. At the end of reperfusion, the rats were sacrificed by decapitation and brains removed, hippocampi isolated and homogenized in ice cold saline for estimation of lactate dehydrogenase activity, oxidative stress markers (thiobarbituric acid reactive substances, total nitrate/nitrite and total antioxidant capacity) and inflammatory biomarkers (myeloperoxidase activity, tumor necrosis factor- α , interleukin-6 and interleukin-10 contents). Resveratrol and vitamin E protected against oxidative stress, lipid peroxidation and inflammation associated with IR injury.

Key words: Vitamin E, resveratrol, ischemia/reperfusion, stroke.

INTRODUCTION

Stroke is a rapidly developing signs of focal or global disturbance of cerebral function (Sudlow and Warlow, 1997). It usually occurs because a blood vessel bursts or is blocked by a clot. When blood flow to the brain is

interrupted an area of the brain is deprived of oxygen and other nutrients (Selman et al., 1997). Given that the brain requires an un-interrupted supply of blood, the longer the duration of cerebral ischemia the lower the chance of

*Corresponding author. E-mail: zaena.khalid@yahoo.com

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reversible injury. The area which is severely affected by the lack of cerebral blood flow is termed the "ischemic core" and cell death of both neurons and astrocytes is profound in this region (Kogure et al., 1992).

Independently from the mechanism responsible for the vessel occlusion, ischemia causes a cascade of events that eventually lead to neuronal damage and death (Fisher and Schaebitz, 2000). The decline of blood flow decreases high energy phosphates production. The energy failure results in membrane depolarization and abandoned release of excitatory aminoacids, such as glutamate, in the extracellular space and excitotoxicity. Glutamate acts on various types of receptors, namely, NMDA and AMPA, eventually causing calcium overload in neuronal cells. Neuronal nitric oxide synthase is also calcium dependent and produces nitric oxide (NO) that plays a crucial role in the course of cerebral ischemia by reacting with superoxide and generating highly reactive radical peroxynitrite (Moro et al., 2004). Secondary to ischemia pro-inflammatory genes are expressed and several inflammatory mediators are released. Currently, there is increasing evidence that inflammation plays a major role in the setting of cerebral ischemia (Tuttolomondo et al., 2012). Experimental studies have shown that inhibition of the inflammatory process has lead to decrease in the extent of injury, an aspect which has gained great importance in understanding and management of stroke (Adibhatla and Hatcher, 2008; Tănăsescu et al., 2008).

Although excitotoxicity typically leads to necrosis, it has been proposed that both apoptosis and necrosis processes are triggered in parallel during ischemia and that the specific conditions determine which one will predominate (Lee et al., 1999).

A wide variety of drugs, which interfere at various points in the ischemic cascade, so-called neuroprotective agents have been used in both experimental and clinical studies. Antioxidants have shown benefits in minimizing the extent of injury and neuronal loss in cerebral ischemia. Treatment of rats with the antioxidants such as vitamin E protects against reactive oxygen species and has decreased the extent of injury in different models of brain ischemia (Knuckey et al., 1995; Sekhon et al., 2003; Jatana et al., 2006).

Resveratrol, a polyphenol phytoalexin, abundantly found in grape skins, has been reported to have multiple physiological effects, including the prevention of lipid peroxidation in human LDL (Frankel et al., 1993), inhibition of a rachidonic acid metabolism (Shin et al., 1998), anti-oxidative (Gedik E1 Girgin et al., 2008; Mozaffarieh et al., 2008) and anti-inflammatory effects (Kubota et al., 2009; Csiszar, 2011). Several studies have shown that and resveratrol reduces the risk of atherosclerosis, renal and cardiovascular diseases (Hao and He, 2004; Raval et al., 2008). These protective actions of resveratrol in cerebral ischemia have so far been identified from studies *in vivo* and in cultured neurons (Ray et al., 1999; Raval et al., 2006; Han et al.,

2004). Although, early effects may be of little relevance to treating clinical stroke; they are necessary for understanding all the mechanisms of action of any potential therapeutic agent and may be beneficial if agents are being developed for prophylactic use. Therefore, the goal of the present study was to explore the protective effects of resveratrol in comparison to vitamin E on cerebral ischemia-reperfusion.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200 to 250 g were obtained from the animal facility of Faculty of Pharmacy, Cairo University. Rats were housed under controlled temperature ($25 \pm 2^\circ\text{C}$) and constant light cycle (12 h light/dark) and allowed free access to a standard rodent chow diet and water. The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

Chemicals

Vitamin E and resveratrol (trans-3,5,4'-Trihydroxystilbene, 99% (GC)) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other used chemicals were of analytical grade.

Experimental design

Rats were randomly divided into five groups, 8 animals each. Group 1: served as a sham operated group in which a midline incision was made and both carotid arteries were exposed for 2 h without occlusion. Group 2: served as ischemic control (I/R) group. Groups 3 and 4: received vitamin E (100 mg/kg) or resveratrol (10 mg/kg), respectively for 14 days and on the 15th day I/R was induced. All drugs were freshly prepared daily in saline, administered orally.

Induction of cerebral ischemia/reperfusion

Rats were anaesthetized with (50 mg/kg thiopental, ip). Hair around the neck was shaved, a midline incision was made and both carotid arteries were exposed. Each carotid artery was freed from its adventitial sheath and vagus nerve (Ulrich et al., 1998). Both arteries were then occluded for 60 min using bull dog clip. Then the carotids were de-clamped and reperfusion was allowed for another 60 min (Seif-EI-Nasr and EI-Fattah, 1995). Temperature was kept constant during and after surgery using a heat lamp placed above the head of the animal in order to prevent cerebral hypothermia (Seif-EI-Nasr et al., 1992).

Biochemical measurements

Following I/R, animals were killed by decapitation, brains were removed and both hippocampi were isolated and homogenized in ice-cold NaCl (0.9%), using a homogenizer (HeidolphDiAx 900, Germany) to prepare 10% homogenate. The resultant homogenates were then used for determination of the following parameters.

Lactate dehydrogenase activity (LDH)

LDH was carried out according to the method described by Buhl and Jackson (1978) using Stanbio kit and expressed as U/g wet weight (Buhl and Jackson, 1978).

Lipid peroxidation products

Lipid peroxidation products were estimated by determination of the level of thiobarbituric acid reactive substances (TBARS) that were measured according to the assay of Buege and Aust (1978) and expressed as nmol/g wet weight.

Tissue total nitrate/nitrite (NO_x)

NO_x was determined spectrophotometrically at 540 nm using Griess reagent after reduction of nitrate to nitrite by vanadium trichloride (Miranda et al., 2001) and expressed as μmol/g wet weight.

Total antioxidant capacity (TAC)

TAC was assessed using rat total antioxidant capacity kit (BIODIAGNOSTIC, Egypt). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/g wet weight.

Tumor necrosis factor-alpha (TNF-α)

TNF-α content was assessed using rat TNF-α ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/g wet weight.

Interleukin-6 (IL-6)

IL-6 was assessed using rat IL-6 ELISA kit (BD Biosciences, San Diego, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/g wet weight.

Interleukin-10 (IL-10)

IL-10 was assessed using rat IL-10 ELISA kit (R&D, USA). The procedure of the used kit was performed according to the manufacturer's instructions and the results were expressed as pg/g wet weight.

Myeloperoxidase activity (MPO)

MPO was determined kinetically at 460 nm by measuring rate of H₂O₂-dependent oxidation of o-dianisidine that is catalyzed by MPO. One unit of MPO activity is defined as the amount of enzyme that degrades 1 μmol peroxide per min at 25°C (Bradley et al., 1982). MPO activity is expressed as mU/g wet weight.

Statistical analysis

All data obtained were presented as mean ± standard error of mean (SEM). Results were analyzed using one way analysis of variance

test (One-way ANOVA) followed by Student-Newman-Keuls multiple comparison test. Statistical analysis was performed using GraphPadInstat software (version 2.04). For all the statistical tests, the level of significance was fixed at p<0.05.

RESULTS

Effect on LDH activity

I/R was associated with elevation in LDH activity about 6 folds that is in the sham operated group. Pre-administration of vitamin E and resveratrol offered neuro-protection in our model of ischemia-reperfusion evident by a reduction in LDH activity to 49.41 and 31.22% of the I/R group, respectively (Figure 1).

Effect on oxidative stress biomarkers

Following induction of I/R, oxidative stress was manifested by a significant increase in lipid peroxidation manifested as 2 fold increase in TBARS content compared to sham operated group. Furthermore, I/R lowered the total antioxidant capacity to be 79.6% of that in sham operated group (Figures 2 and 3). Total nitrate/nitrite (NO_x) content was elevated almost 2 folds of that in the sham operated group. Pre-treatment with vitamin E or resveratrol reduced TBARS to be 77.64 and 78.84% of I/R group, respectively. Meanwhile, NO_x content was reduced only in vitamin E to be 62.76% of I/R group (Figure 4).

Effect on inflammatory biomarkers

Following induction of I/R inflammation was manifested by about 2-fold increase in TNF-α, IL-6 contents (Figures 5 and 6), together with a significant decrease in IL-10 content of 28.6% of the sham operated group (Figure 7). Pre-treatment with vitamin E or resveratrol reduced TNF-α to be 70 and 75% of the I/R group, respectively. Meanwhile, IL-6 was reduced to be 45.65 and 69.56% in vitamin E and resveratrol groups, respectively. On the other hand, both drugs elevated IL-10 to be 182.65 and 269.86% of the I/R group. I/R induced MPO activity to be 158.82% of the sham operated group and effect that was counteracted by pretreatment with vitamin E or resveratrol where MPO activity reached 74.07 and 59.25% in the aforementioned groups, respectively (Figure 8).

DISCUSSION

Interruption of cerebral blood flow leads to vascular leakage, inflammation, tissue injury, and necrosis (Wexler, 1970; Martins et al., 1980). Changes associated with ischemia include impairment of metabolism, energy

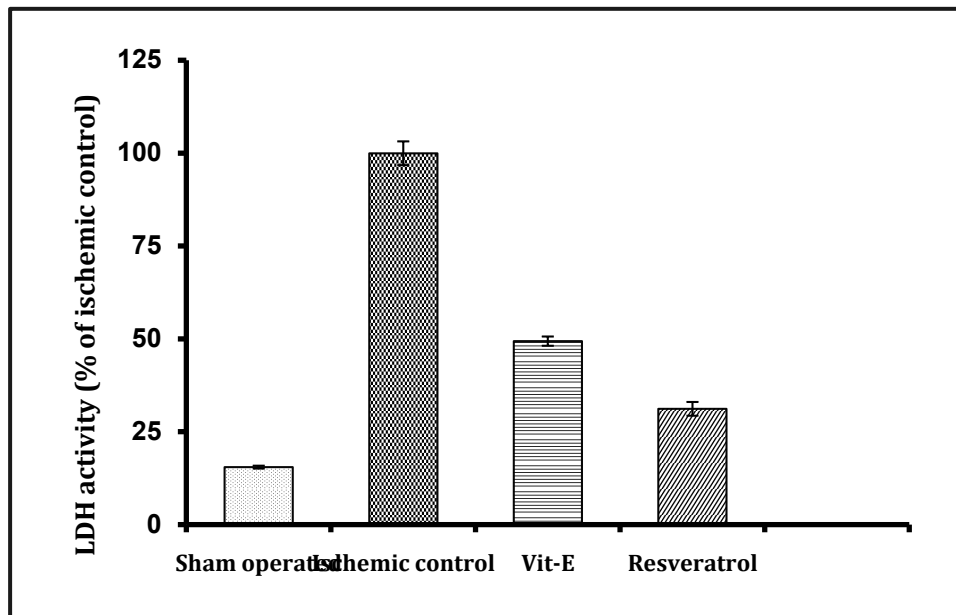


Figure 1. Effect of resveratrol and vitamin-E (Vit-E) on lactate dehydrogenase (LDH) activity in the hippocampus tissue in rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed.

Data was expressed as means \pm S.E. and as percent of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by the Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

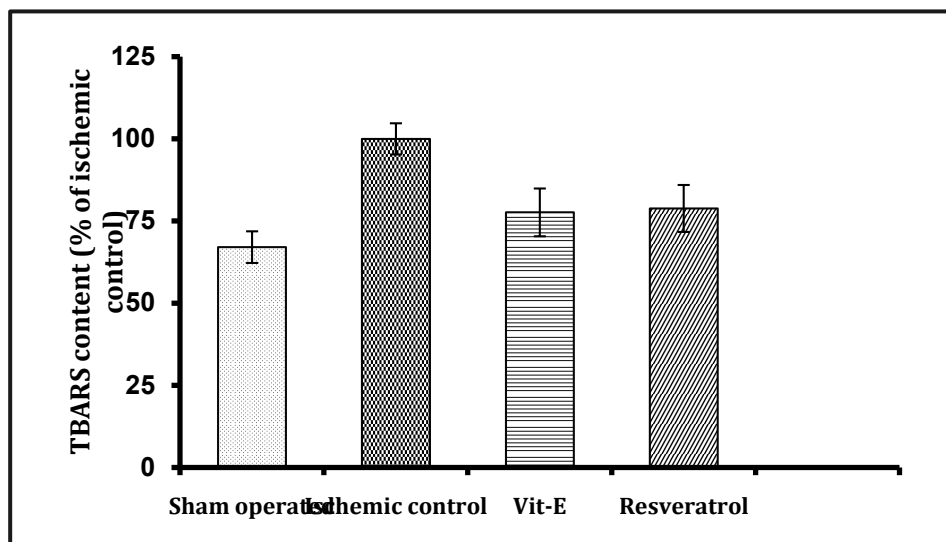


Figure 2. Effect of resveratrol and vitamin-E (Vit-E) on thiobarbituric acid reactive substances (TBARS) content in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed.

Data was expressed as means \pm S.E. and as % of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups.

*Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

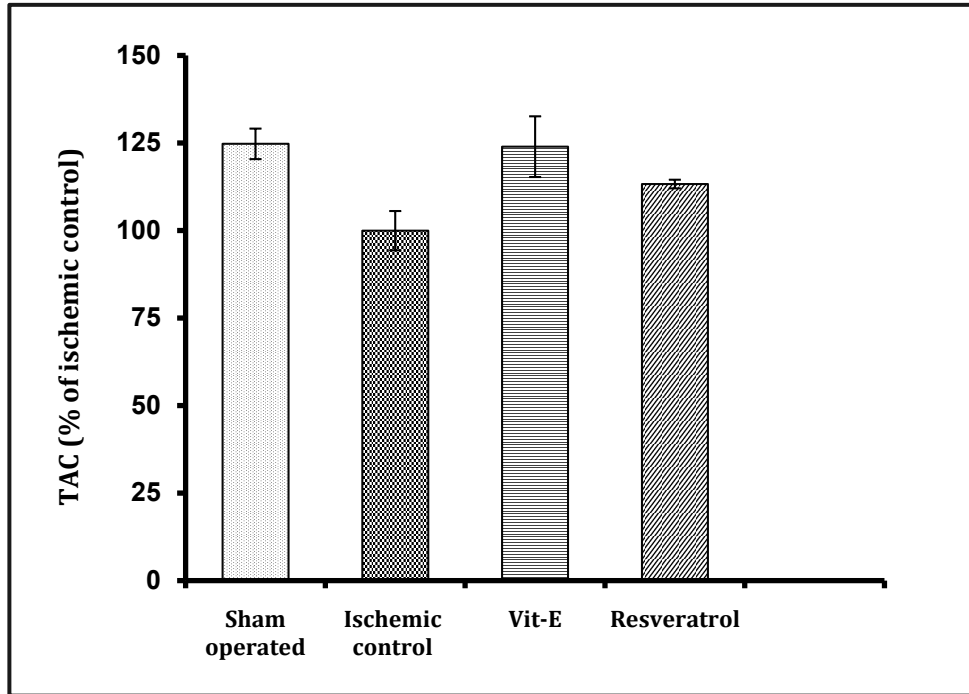


Figure 3. Effect of resveratrol and vitamin-E (Vit-E) on total antioxidant capacity (TAC) in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, n = 8. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at p < 0.05. @Significantly different from ischemic control group at p < 0.05.

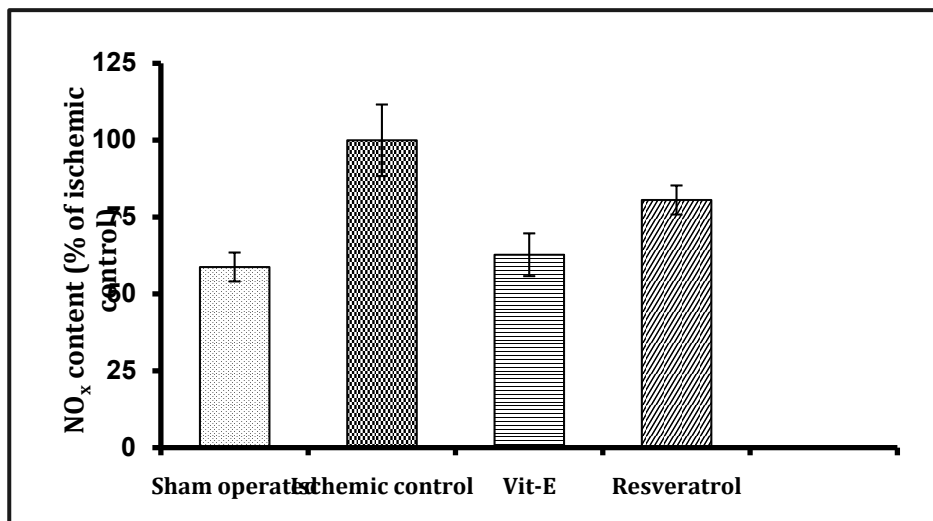


Figure 4. Effect of resveratrol and vitamin-E (Vit-E) on total nitrate/nitrite (NO_x) content in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, n = 8. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at p < 0.05. @Significantly different from ischemic control group at p < 0.05.

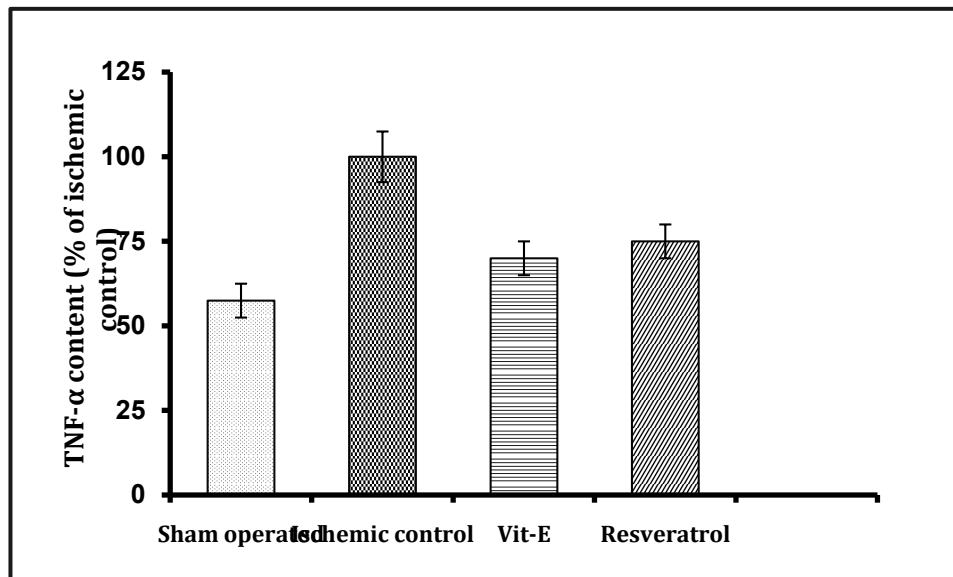


Figure 5. Effect of resveratrol and vitamin-E (Vit-E) on tumor necrosis factor-alpha (TNF- α) content in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

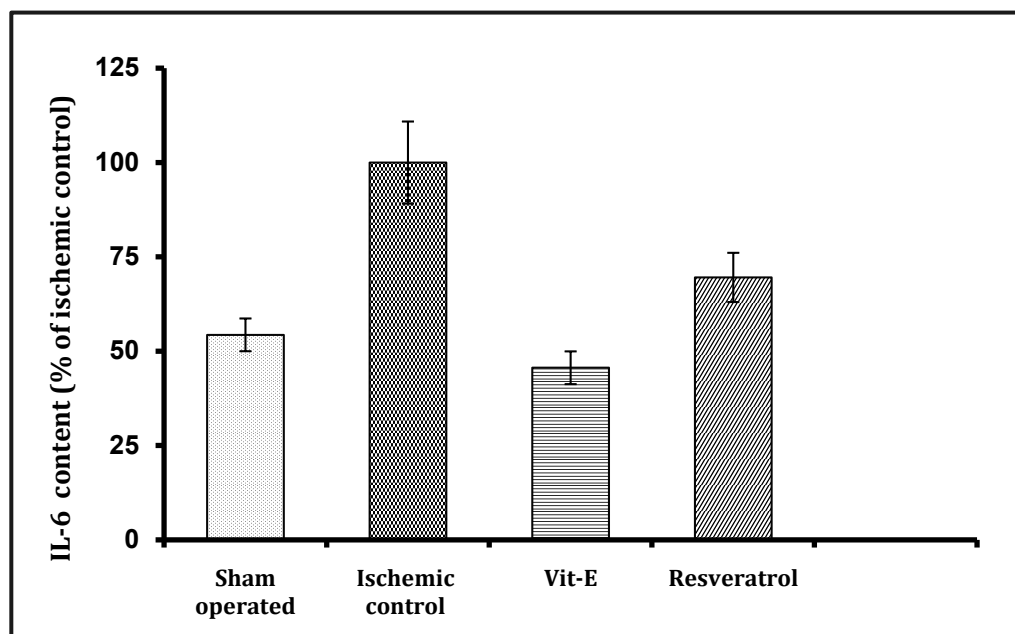


Figure 6. Effect of resveratrol and vitamin-E (Vit-E) on interleukin-6 (IL-6) content in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

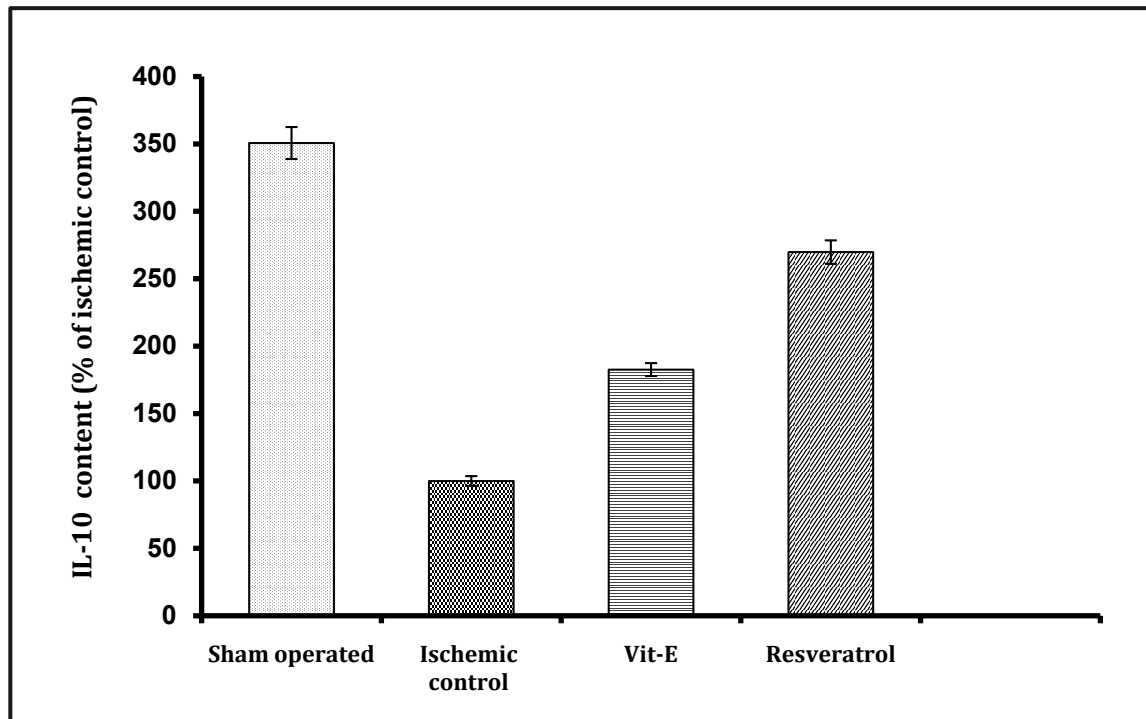


Figure 7. Effect of resveratrol and vitamin-E (Vit-E) on interleukin-10 (IL-10) content in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

failure, free radical production, excitotoxicity, altered calcium homeostasis, and activation of proteases (Choi, 1980; Panickar and Norenberg, 2005). Cerebral ischemia also turns down in the ability of brain mitochondria to function effectively thus affecting oxidative phosphorylation, a key mechanism of producing adenosine triphosphate (ATP).

Mitochondrial dysfunction may contribute to increased reactive oxygen species (ROS) production and lead to depolarization of the inner mitochondrial membrane potential. Such ischemia-associated changes can contribute to Ca^{2+} -induced membrane damage as well as increases in the Ca^{2+} -induced proteases, free radical mediated cell damage including membrane lipid peroxidation, and DNA damage (Sims and Muyderman, 2010). Minimizing oxidative stress and mitochondrial damage may result in reduced cell damage and a consequent improvement in cell viability following cerebral ischemia.

Increasing evidence supports the hypothesis that plant polyphenols provide protection against neurodegenerative changes associated with cerebral ischemia (Simonyi et al., 2005). Phenolic antioxidants have been shown to inhibit oxidative stress, a key event in the pathogenesis of cerebral ischemia, that damage

lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. Most studies have reported the protective effects of polyphenols in the hippocampal and cerebral cortex regions in ischemia (Dajas et al., 2003; Halliwell, 2008).

Levels of LDH activity could act as the marks of energy metabolism changes in the injured tissues and their quantity and quality could directly affect body's energy metabolism. When tissues and organs were injured, LDH activity abnormally increased (Rodrigo and Bosco, 2006; Doganay et al., 2006). In the present experimental results, resveratrol reduced LDH activity an effect that can be clarified by that tissue injury and cell death due to I/R is a free radical-mediated process in which oxidative stress and lipid peroxidation results in loss of cell viability and increased LDH activity. Resveratrol had a protective effect against LDH increasing and lipid peroxidation (Chander et al., 2005). It was also reported that a resveratrol analogue, astringinin, strongly prevented myocardial ischemia and infarction through increasing nitric oxide (NO) and decreasing LDH levels in the carotid blood (Luzi et al., 2004). Therefore, resveratrol may play an important role in protection against I/R through the observed improvement of LDH activity after tissue trauma.

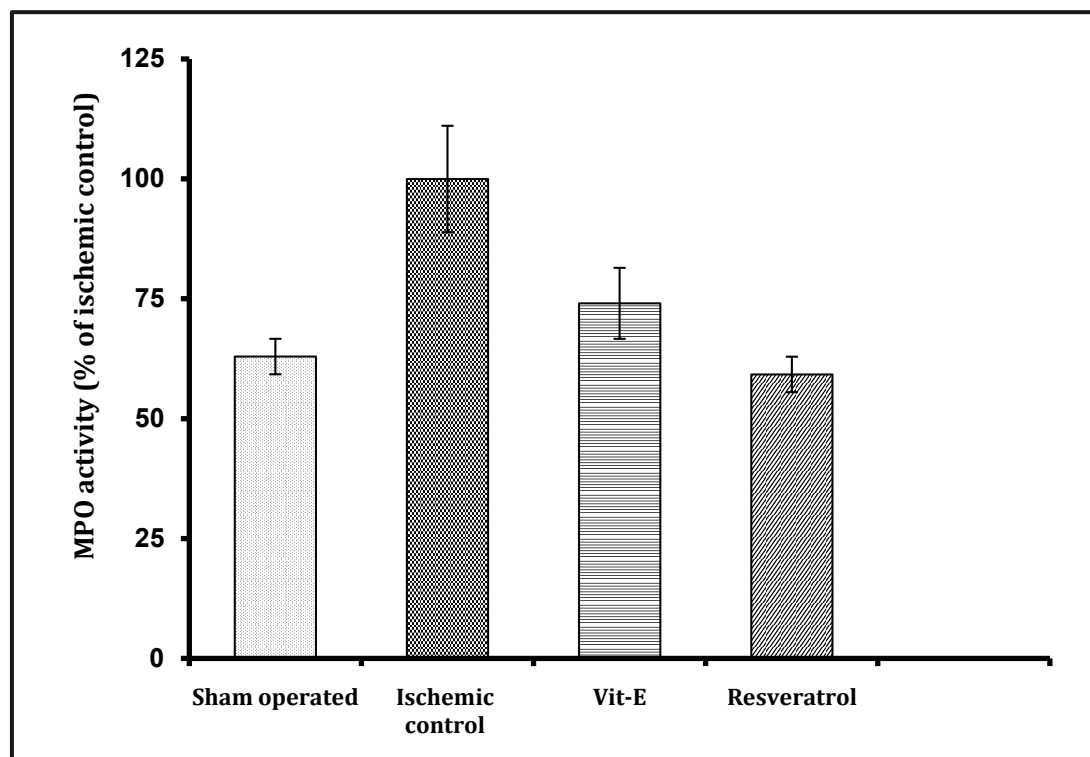


Figure 8. Effect of resveratrol and vitamin-E (Vit-E) on myeloperoxidase (MPO) activity in the hippocampus tissue of rats subjected to Ischemia/Reperfusion (I/R) injury. All drugs were administered for 14 consecutive days, and on the 15th day ischemia followed by reperfusion were performed. Data was expressed as means \pm S.E. and as % of I/R control, $n = 8$. Statistical analysis was carried out by one-way ANOVA followed by The Tukey Kramer multiple comparisons test for comparison of means of different groups. *Significantly different from sham operated group at $p < 0.05$. @Significantly different from ischemic control group at $p < 0.05$.

In the present work, the antioxidant potential of both vitamin E and resveratrol was shown through a decrease in lipid peroxidation measured as TBARS content. Vitamin E was found to reduce the volume of ischemic infarct (Garcia-Estrada et al., 2003; Mishima et al., 2003). In addition, administration of vitamin E alone or in combination with other vitamins increases the activities of antioxidant enzymes in rats following cerebral ischemia (Kashif et al., 2004). Gümüştaş et al. (2007) showed that administration of vitamin E before cerebral ischemia reduced lipid peroxidation and nitric oxide production in rats through free radicals scavenge properties of vitamin E (Gümüştaş et al., 2007).

Furthermore, resveratrol has been recently proposed as a potential antioxidant that could obviously inhibit free radical generation in several body tissues (Lu et al., 2013). In addition, the experiments *in vitro* also demonstrated that resveratrol could effectively protect DNA from oxidative damages, so as to assure cell proliferation, differentiation, and function to be normal (Jeong et al., 2014). Ray et al. (1999) found that resveratrol possessed cardio-protective effects through its peroxyl radical scavenging activity and inhibiting lipid peroxidation (Ray et al., 1999).

Total antioxidant capacity was not altered in the resveratrol-treated group; this can be attributed to the fact that this parameter measures the combined enzymatic and non-enzymatic antioxidants capacity of the biological fluids. Thus, it provides an indication of the overall capability to counteract reactive oxygen species (ROS), resist oxidative damage and combat oxidative stress-related diseases. In some cases, the antioxidant effect may be mediated by counteracting the effect of certain enzyme which may potentiate the effect of another and thus the result is a non significant change.

I/R and associated oxidative stress up-regulates the expression of iNOS and increase NO_x concentrations (Huang et al., 2011). NO_x is synthesized during the stoichiometric conversion of L-arginine to L-citrulline in the presence of oxygen and nicotinamide adenine dinucleotide phosphate (NADPH), which is catalyzed by NOS (Moncada et al., 1991). Our results have indicated that I/R increased NO_x content in the hippocampus. This elevated NO_x content was not altered following resveratrol administration an effect that can be explained that shows that resveratrol up-regulates the expression of iNOS, indicating the ability of resveratrol to induce eNO synthesis. In some tissue assays, resveratrol was found

to exert its protective action through up-regulation of NO synthesis thus it failed to neutralize the elevation of NO_x following I/R (Chander et al., 2005; Luzi et al., 2004).

In the present investigation, Global I/R was associated with inflammation indicated by elevation in both TNF- α and IL-6 hippocampal contents. Inflammatory activity is associated with increased levels of pro-inflammatory cytokines in the circulation, including IL-6 and TNF α (Bruunsgaard et al., 2001; Calabro et al., 2008). IL-6, along with TNF- α and IL-1 β , is known to play a key role in B-cell maturation and T-cell differentiation, as well as driving acute inflammatory responses (Schuett et al., 2012).

It has been observed that vitamin E suppresses inflammatory responses and oxidative damage induced by lipo-polysaccharide (LPS), a highly conserved cell wall component of Gram-negative bacteria known to initiate signaling cascade for inflammatory mediator expression including TNF- α , IL-6, and nuclear factor-kappaB, in both cell culture systems and animal experiments (Suntres and Shek, 1996; Takata et al., 1997; Berg et al., 2004). It was reported that α -tocopherol effectively prevented interferon-gamma/LPS-induced dopaminergic neuron degeneration (Shibata et al., 2006) and decreased LPS-induced lipid peroxidation and IL-6 in murine microglia and brain (Godbout et al., 2004).

Resveratrol reduced both TNF- α and IL-6 hippocampal content. Chen et al. (2013) demonstrated that resveratrol reduced joint swelling and cartilage destruction in adjuvant arthritis through inhibition of TNF- α production and showed that resveratrol down regulated the mRNA expression levels of the inflammatory factor, TNF- α (Chen et al., 2013). Furthermore, it was shown that treatment of diabetic rats with resveratrol significantly depressed TNF- α and IL-6 transcripts and the nuclear translocation of NF- κ B, suggesting an anti-inflammatory effect of resveratrol in the brain additionally. The anti-inflammatory effect of resveratrol has been studied in many other organs, including liver, heart, lung and kidneys (De la Lastra and Villegas, 2005; Docherty et al., 2005; Migliore and Coppede, 2009; Norata et al., 2007).

Conclusion

Briefly, this study demonstrates the beneficial influences of resveratrol on cerebral I/R through its antioxidant properties and inhibition of lipid peroxidation as well as through its anti inflammatory potentials.

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Conflicts of interest

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REFERENCES

- Adibhatla RM, Hatcher JF (2008). Phospholipase A2, reactive oxygen species, and lipid peroxidation in CNS pathologies. *BMB Rep.* 41(8):560-567.
- Berg BM, Godbout JP, Kelley KW, Johnson RW (2004). Alpha-tocopherol attenuates lipopolysaccharide-induced sickness behavior in mice. *Brain Behavior and Immunity* 18:149-157.
- Bradley PP, Christensen RD, Rothstein G (1982). Cellular and extracellular myeloperoxidase in pyogenic inflammation. *Blood* 60(3):618-622.
- Bruunsgaard H, Pedersen M, Pedersen BK (2001). Aging and proinflammatory cytokines. *Curr. Opin. Hematol.* 8:131-136.
- Buege JA, Aust SD (1978). Microsomal lipid peroxidation. *Methods Enzymol.* 52:302-310.
- Buhl SN, Jackson KY (1978). Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 degrees C. *Clin. Chem.* 24(5):828-831.
- Calabro P, Limongelli G, Pacileo G, Di Salvo G, Golino P, Calabro R (2008). The role of adiposity as a determinant of an inflammatory milieu. *J. Cardiovasc. Med.* 9:450-460.
- Chander V, Tirkey N, Chopra K (2005). Resveratrol, a polyphenolic phytoalexin protects against cyclosporine-induced nephrotoxicity through nitric oxide dependent mechanism. *Toxicology* 210:55-64.
- Chen XY, Wang ZC, Li J, Liu XL, Sun YH (2013). Regulation of synovioocyte activity by resveratrol in rats with adjuvant arthritis. *Exp. Ther. Med.* 6:172-176.
- Choi DW (1988). Calcium-mediated neurotoxicity: Relationship to specific channel types and role in ischemic damage. *Trends Neurosci.* 11:465-469.
- Csiszar A (2011). Anti-inflammatory effects of resveratrol: possible role in prevention of age-related cardiovascular disease. *Ann. NY Acad. Sci.* 1215:117-122.
- Dajas F, Rivera-Megret F, Blasina F, Arredondo F, Abin-Carriquiry JA, Costa G, Echeverry C, Lafon L, Heizen H, Ferreira M (2003). Neuroprotection by flavonoids. *Braz. J. Med. Biol. Res.* 36:1613-1620.
- De la Lastra CA, Villegas I (2005). Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Mol. Nutr. Food Res.* 49:405-430.
- Docherty JJ, Fu MM, Hah JM, Sweet TJ, Faith SA, Booth T (2005). Effect of resveratrol on herpes simplex virus vaginal infection in the mouse. *Antiviral Res.* 67:155-162.
- Doganay S, Borazan M, Iraz M, Cigremis Y (2006). The effect of resveratrol in experimental cataract model formed by sodium selenite. *Curr. Eye Res.* 31:147-153.
- Fisher M, Schaebitz W (2000). An overview of acute stroke therapy: past, present, and future. *Arch. Intern. Med.* 160(21):3196-3206.
- Frankel EN, Waterhouse AL, Kinsella JE (1993). Inhibition of human LDL oxidation by resveratrol. *Lancet.* 341:1103-1104.
- Garcia-Estrada J, Gonzalez-Perez O, Gonzalez-Castaneda RE, Martinez-Contreras A, Luquin S, de la Mora PG, Navarro-Ruiz A (2003). An alpha-lipoic acid-vitamin E mixture reduces postembolism lipid peroxidation, cerebral infarction, and neurological deficit in rats. *Neurosci. Res.* 47:219-224.
- Gedik E1, Girgin S, Ozturk H, Obay BD, Ozturk H, Buyukbayram H (2008). Resveratrol attenuates oxidative stress and histological alterations induced by liver ischemia/reperfusion in rats. *World J. Gastroenterol.* 14(46):7101-7106.
- Godbout JP, Berg BM, Kelley KW, Johnson RW (2004). Alpha-Tocopherol reduces lipopolysaccharide-induced peroxide radical

- formation and interleukin-6 secretion in primary murine microglia and in brain. *J. Neuroimmunol.* 149:101-109.
- Gümüştaş K, Meta Güzeyli FM, Atükere P, Sanus GZ, Kemerdere R, Tanriverdi T, Kaynar MY (2007). The effects of vitamin E on lipid peroxidation, nitric oxide production and superoxide dismutase expression in hyperglycemic rats with cerebral ischemia-reperfusion injury. *Turk. Neurosurg.* 17(2):78-82.
- Halliwell B (2008). Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch. Biochem. Biophys.* 476:107-112.
- Han YS, Zheng WH, Bastianetto S, Chabot JG, Quirion R (2004). Neuroprotective effects of resveratrol against beta-amyloid-induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. *Br. J. Pharmacol.* 141(6):997-1005.
- Hao HD, He LR (2004). Mechanisms of cardiovascular protection by resveratrol. *J. Med. Food.* 7(3):290-298.
- Huang TC, Lu KT, Wo YY, Wu YJ, Yang YL (2011). Resveratrol protects rats from a beta-induced neurotoxicity by the reduction of iNOS expression and lipid peroxidation. *Plos One* 6(12):e29102.
- Jatana M, Singh I, Singh AK, Jenkins D (2006). Combination of systemic hypothermia and N-acetylcysteine attenuates hypoxic-ischemic brain injury in neonatal rats. *Pediatr Res.* 59:684-689.
- Jeong MH, Yang KM, Jeong DH, Lee CG, Oh SJ, Jeong SK, Lee KW, Jo YR, Jo WS (2014). Protective activity of a novel resveratrol analogue, HS-1793, against DNA damage in 137Cs-irradiated CHO-K1 cells. *J. Radiat. Res.* [Epub ahead of print].
- Kashif R, Zaidi SM, Banu N (2004). Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. *Clin. Chim. Acta.* 340:229-233.
- Knuckey NW, Palm D, Primiano M, Epstein MH, Johanson CE (1995). N-acetylcysteine enhances hippocampal neuronal survival after transient forebrain ischemia in rats. *Stroke* 26:305-310.
- Kogure K, Watson BD, Busto R, Abe K (1992). Potentiation of lipid peroxides by ischemia in rat brain. *Neurochem. Res.* 7(4):437-454.
- Kubota S, Kurihara T, Mochimaru H, Satofuka S, Noda K, Ozawa Y, Oike Y, Ishida S, Tsubota K (2009). Prevention of ocular inflammation in endotoxin-induced uveitis with resveratrol by inhibiting oxidative damage and nuclear factor-kappaB activation. *Invest. Ophthalmol. Vis. Sci.* 50(7):3512-3519.
- Lee JM, Zipfel GJ, Choi DW (1999). The changing landscape of ischaemic brain injury mechanisms. *Nature* 399(6738 Suppl):A7-14.
- Lu X, Xu H, Sun B, Zhu Z, Zheng D, Li X (2013). Enhanced neuroprotective effects of resveratrol delivered by nanoparticles on hydrogen peroxide-induced oxidative stress in rat cortical cell culture. *Mol. Pharm.* 10(5):2045-2053.
- Luzi C, Brisdelli F, Cinque B, Bozzi GCA (2004). Differential sensitivity to resveratrol-induced apoptosis of human chronic myeloid (K562) and acute lymphoblastic (HSB-2) Leukemia cells. *Biochem. Pharmacol.* 68:2019-2030.
- Martins E, Inamura K, Themner K, Malmqvist KG, Siesjö BK (1988). Accumulation of calcium and loss of potassium in the hippocampus following transient cerebral ischemia: A proton microprobe study. *J. Cereb. Blood Flow Metab.* 8:531-538.
- Migliore L, Coppede F (2009). Environmental-induced oxidative stress in neurodegenerative disorders and aging. *Mutat. Res.* 674:73-84.
- Mishima K, Tanaka T, Pu F, Egashira N, Iwasaki K, Hidaka R, Matsunaga K, Takata J, Karube Y, Fujiwara M (2003). Vitamin E isoforms --tocotrienol and α -tocopherol prevent cerebral infarction in mice. *Neurosci. Lett.* 337:56-60.
- Moncada S, Palmer RM, Higgs EA (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109-142.
- Moro MA, Cárdenas A, Hurtado O, Leza JC, Lizasoain I (2004). Role of nitric oxide after brain ischaemia. *Cell Calcium* 36(3-4):265-275.
- Mozaffarieh M, Grieshaber MC, Orgül S, Flammer J (2008). The potential value of natural antioxidative treatment in glaucoma. *Surv. Ophthalmol.* 53(5):479-505.
- Norata GD, Marchesi P, Passamonti S, Pirillo A, Violi F, Catapano AL (2007). Anti-inflammatory and anti-atherogenic effects of catechin, caffeic acid and trans-resveratrol in apolipoprotein E deficient mice. *Atherosclerosis* 191:265-271.
- Panickar KS, Norenberg MD (2005). Astrocytes in cerebral ischemic injury: Morphological and general considerations. *Glia.* 50:287-298.
- Raval AP, Dave KR, Pérez-Pinzón MA (2006). Resveratrol mimics ischemic preconditioning in the brain. *J. Cereb. Blood Flow Metab.* 26(9):1141-1147.
- Raval AP, Lin HW, Dave KR, Defazio RA, Della Morte D, Kim EJ, Perez-Pinzón MA (2008). Resveratrol and ischemic preconditioning in the brain. *Curr. Med. Chem.* 15(15):1545-1551.
- Ray PS, Maulik G, Cordis GA, Bertelli AA, Bertelli A, Das DK (1999). The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radic. Biol. Med.* 27(1-2):160-169.
- Rodrigo R, Bosco C (2006). Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. *Biochem. Physiol.* 142:317-327.
- Schuetz H, Oestreich R, Waetzig GH, Annema W, Luchtefeld M, Hillmer A, Bavendiek U, von Felden J, Divchev D, Kempf T (2012). Transsignaling of interleukin-6 crucially contributes to atherosclerosis in mice. *Arteriosclerosis* 32:281-290.
- Seif-El-Nasr M, El-Fattah AA (1995). Lipid peroxide, phospholipids, glutathione levels and superoxide dismutase activity in rat brain after ischaemia: effect of *Ginkgo biloba* extract. *Pharmacol. Res.* 32:273-278.
- Seif-El-Nasr NM, Nuglisch J, Kriegelstein J (1992). Prevention of ischemia-induced cerebral hypothermia by controlling the environmental temperature. *J. Pharmacol. Toxicol. Methods.* 27:23-26.
- Sekhon B, Sekhon C, Khan M, Patel SJ, Singh I, Singh AK (2003). N-Acetyl cysteine protects against injury in a rat model of focal cerebral ischemia. *Brain Res.* 971:1-8.
- Selman WR, Tarr R, Landis DM (1997). Brain attack: emergency treatment of ischemic stroke. *Am. Fam. Physician.* 55:2655-2656.
- Shibata H, Katsuki H, Okawara M, Kume T, Akaike A (2006). c-JunN-terminal kinase inhibition and alpha-tocopherol protect mid brain dopaminergic neurons from interferon-gamma/lipopolysaccharide-induced injury without affecting nitric oxide production. *J. Neurosci. Res.* 83:102-109.
- Shin NH, Ryu SY, Lee H, Min KR, Kim Y (1998). Inhibitory effects of hydroxystilbenes on cyclooxygenase from sheep seminal vesicles. *Planta. Med.* 64(3):283-284.
- Simonyi A, Wang Q, Miller RL, Yusuf M, Shelat PB, Sun AY, Sun GY (2005). Polyphenols in cerebral ischemia: novel targets for neuroprotection. *Mol. Neurobiol.* 31:135-147.
- Sims NR, Muyderman H (2010). Mitochondria, oxidative metabolism and cell death in stroke. *Biochim. Biophys. Acta.* 1802:80-91.
- Sudlow CL, Warlow CP (1997). Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. *International Stroke Incidence Collaboration.* *Stroke* 28:491-499.
- Suntres ZE, Shek PN (1996). Treatment of LPS-induced tissue injury: role of liposomal antioxidants. *Shock* 6(1):S57-S64.
- Takata J, Ito S, Karube Y, Nagata Y, Matsushima Y (1997). Water-soluble prodrug of vitamin E for parenteral use and its effect on endotoxin-induced liver toxicity. *Biol. Pharm. Bull.* 20:204-209.
- Tănăsescu R, Nicolau A, Ticmeanu M, Luca D, Caraiola S, Cojocaru IM, Frăsineanu A, Ionescu R, Hristea A, Ene A, Tănăsescu R, Baicuș C (2008). An immunological approach to cerebral ischemia (I). Immune cells and adhesion molecules. *Rom. J. Intern. Med.* 46(1):3-8.
- Tuttolomondo A, Di Raimondo D, Pecoraro R, Arnao V, Pinto A, Licata G (2012). Inflammation in ischemic stroke subtypes. *Curr. Pharm. Des.* 18(28):4289-4310.
- Ulrich PT, Kroppenstedt S, Heimann A, Kempfski O (1998). Laser-doppler scanning of local cerebral blood flow and reserve capacity and testing of motor and memory function in a chronic 2-vessel occlusion model in rats. *Stroke* 29:2412-2420.
- Wexler BC (1970). Metabolic changes in response to acute cerebral ischemia following bilateral carotid artery ligation in arteriosclerotic versus nonarteriosclerotic rats. *Stroke* 1:112-121.



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