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CLINICAL STUDY

Preeclamptic cord blood hemolysis and the effect of *Monascus purpureus* and *Saccharomyces cerevisiae* in modulating preeclamptic stress

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Abstract: *Background:* Preeclampsia is associated with impaired antioxidant defense that results in materno-fetal complications. In addition to antioxidant deficiency, hemolytic disorder has also been observed in pre-eclamptic mother.
Methods: This study aims in analyzing the fetal complications using cord blood RBC (red blood cell); further the antihemolytic and antioxidant efficiency of two common probiotic yeasts *Monascus purpureus* and *Saccharomyces cerevisiae* in preeclamptic and normotensive RBCs were assessed.
Results: There was a significant decrease in the antioxidant status ($p<0.05$) with increased oxidative stress, nitrative stress ($p<0.05$) and hemolysis ($p<0.001$) in preeclamptic RBC comparatively. *M. purpureus* demonstrated a highly significant reactive oxygen radical scavenging activity ($p<0.001$) whereas *S. cerevisiae* exhibited a highly significant nitric oxide radical scavenging activity ($p<0.001$). It was noted that oxidative stress hemolysis was decreased with increased antioxidant level in cord blood RBC from both samples after incubation with both yeasts in a similar manner. The antihemolytic property of *M. purpureus* and *S. cerevisiae* suggests that *S. cerevisiae* functions efficiently with increasing stress.
Conclusion: This study demonstrates for the first time that despite their differential scavenging activities, a diet rich in *M. purpureus* and *S. cerevisiae* could equally serve as a good natural supplement to alleviate the stress status in the preeclamptic fetus *(Tab. 4, Fig. 1, Ref. 39)*. Full Text in PDF *www.elis.sk.*
Key words: Cord blood RBC, hemolysis, *Monascus purpureus,* Preeclampsia, *Saccharomyces cerevisiae.*

Abbreviations: RBC – Red blood cell, HBSS – Hank's Balanced Salt Solution, HELLP – Hemolytic anemia Elevated Liver enzymes and Low Platelet count, DPPH – 2,2-diphenyl-1-picrylhydrazyl, MDA – Malondialdehyde, G6PDH – Glucose 6-phosphate dehydrogenase, H_2O_2 – Hydrogen peroxide, TAC – Toatal antioxidant capacity, ABTS 2, – 2'-azinobis-3-ethyl-benzothiozoline-6-sulphonic acid, ROS – Reactive oxygen species, RNS – Reactive nitrogen species, NO_2^- – Nitrite, NO_3^- – Nitrate, IC_{50} – half maximal inhibitory concentration, LPO – Lipid peroxide.

Preeclampsia is a pregnancy-specific condition characterized by hypertension and proteinuria that remits after delivery (1). It is a hypoxic condition (2), which can increase the number of RBC in cord blood (3). Umbilical cord blood RBCs (red blood cells) play a vital role in nourishing the fetus (3). The course of preeclamptic/eclamptic patients may be complicated by HELLP syndrome, syndrome of intravascular hemolysis (H), elevated liver enzymes (EL) and low platelet count (LP) (4). A case of severe preeclampsia in which hemolysis and rapid platelet consumption persisted after delivery was noted in early 1985 (5). Hemolytic disorder like the HELLP syndrome has also been observed in preeclamptic mother in addition to antioxidant deficiency (6), but similar complication in fetus has not been observed.

Preeclamptic stress in the fetus and the corresponding fetal response are reflected in the cord blood. Oxidative stress increases during preeclampsia and results in increased production of lipid peroxides (LPO), reactive oxygen species (ROS) and superoxide anion radicals (7, 8). Antioxidants such as carotenoids, tocopherols, and ascorbic acid are lower in women with preeclampsia (9). In this context, this study was undertaken to determine the changes in cord blood levels of hemolysis, oxidative stress, nitrative stress and antioxidant levels of RBC in preeclamptic subjects compared to normotensive subjects; for analyzing the fetal complication along with its response to preeclampsia. Various natural alternative medicines have been suggested and utilized for controlling preeclampsia.

Yeast contains a system of antioxidant enzymes that protects them from oxidative injury. *Monascus purpureus* and *Saccharomyces cerevisiae* are two most common yeasts having nutritional and medicinal properties more often consumed on the Asian continent. *M. purpureus* is used as medicinal food to improve blood circulation. It is used to prepare fermented red yeast rice which is commonly consumed in many Asian countries (10, 11). Other applications for *M. purpureus* are that lovastatin and other statin drugs produced from this yeast may be useful for treating or pre-

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venting osteoporosis (12, 13). It contains Monacolin K, known as lovastatin, which is a commonly prescribed lipid-lowering drug that significantly reduces high cholesterol. *M. purpureus* contains a large amount of GABA and possesses antihypertensive effects for humans (14). *S. cerevisiae* is alternatively regarded as nutritional yeast. *S cerevisiae* is also found in many commonly consumed Asian food like Dosa, butter, curd, fruit juice and rice cakes (15). *S. cerevisiae* has been studied extensively as a dietary supplement due to the rich source of vitamins and minerals present in it (16). The antioxidant and anti-hemolytic potential of yeast extract was determined through scavenging assays and its ability to inhibit free radical formation through incubation studies with cord blood RBC.

Materials and methods

Selection of sample

About 20 patients with preeclampsia in the age group of 20–40 years were included in the study as test subjects, and 20 normotensive pregnant women of similar age and race were included as control subjects. Patients with preeclampsia were defined on the basis of the following clinical and laboratory criteria (Tab. 1); systolic pressure ≥140 mmHg and diastolic pressure ≥90 mmHg noted at least on two occasions, proteinuria levels ≥300 mg/dl found at least in two random specimens. The control group women were without any maternal and fetal complication during their pregnancy period. There were 5 vaginal deliveries and 15 Caesarean sections in the test group, and 16 vaginal deliveries and 4 Caesarean sections in the control group.

Collection of sample

Immediately after the birth of the baby, the umbilical cord was clamped 8–10 inches away from the first clamp and the cord was cut to collect the cord blood. The blood was collected in a sterile heparin-coated vacutainer.

Tab. 1. Demographic data and clinical characteristics of the normotensive and preeclamptic subjects.

Character	Control (n=20)	Test (n=20)
Maternal age (years)	24.7±2.7	21.5±1.4
Gestational age at delivery (weeks)	37.8±0.4	60.8±4.8**
Pre pregnancy weight (Kg)	50.1±4.2	60.8±4.8*
Pregnancy weight (Kg)	54.3±7.6	69.1±8.1*
Pre pregnancy BP (mmHg)		
Systolic	112.5±6.2	114.5±6.4^{NS}
Diastolic	77.2±5.4	77.1±4.8^{NS}
Pregnancy BP at 20 weeks (mmHg)		
Systolic	115.7±7.5	138.4±4.1*
Diastolic	77.5±4.1	100.52±9.2*
Pregnancy BP at term (mmHg)		
Systolic	120.6±8.0	150.1±8.1*
Diastolic	80.5±6.9	109.2±7.1*
Parity	1.7±0.86	1.3±0.51^{NS}
Type of delivery		
Vaginal	16	5
Caesarean	4	15
Infant birth weight	3.35±0.25	2.51±0.69*

** p<0.05 when compared with RBC isolated from normotensive pregnant women
* p<0.01 when compared with RBC isolated from normotensive pregnant women
NS – not significant when compared with RBC isolated from normotensive pregnant women

Isolation of RBC

An open syringe, filled with cord blood was closed at the tip, kept upright, and then centrifuged at 800g for 20 min at 4 °C without the piston. The resulting plasma was discarded. After opening the syringe at the tip, about 2/3 of RBC sediment was carefully allowed to drop out of the syringe and was used for further analysis. The isolated RBCs were confirmed by performing Glucose 6-phosphate dehydrogenase enzyme assay by the method of Noltmann et al. (17).

Yeast preparation

The white yeast culture was purchased from a private research laboratory, Chennai, India. The microorganism was maintained by sub-culturing in Potato Dextrose agar slants. The Microbial type culture collection strain (MTCC) of red yeast (MTCC No. 369), was purchased from Indian Institute of Microbial Technology, Chandigarh, India. The fungus was maintained by sub-culturing in potato dextrose agar plates.

Incubation studies of RBC with yeast

100 µl of RBCs collected from normotensive and preeclamptic women were incubated with 1 mL of Hanks Balanced Salt Medium containing 5.5 mM glucose (pH 7.4) for 24 h at 37 °C in a shaking water bath. The RBCs were then incubated with *M. purpureus* and *S. cerevisiae* at appropriate concentration for 2 h at 37 °C. Control RBCs were maintained with an equal volume of HBSS (Hank's Balanced Salt Solution) for the same duration at 37 °C.

RBC hemolysis in vitro

This was measured after addition of hydrogen peroxide (H_2O_2) with a technique modified from that described by Horwitt et al (18). A volume of 0.1 mL of RBC was taken in four test tubes. The first tube treated with HBSS serves as a negative control. The second tube treated with 2 % H_2O_2 serves as the positive control. Tubes 3–4 were treated with 0.1 mL of H_2O_2 and 0.1 mL of test samples (RBC incubated with *M. purpureus* and *S. cerevisiae*). All the tubes were incubated at 37 °C for 3 h. All samples were centrifuged, and the absorbance of the supernatant was measured at 575 nm as an indication of the degree of hemolysis.

Determination of antioxidant status of yeast

The activity of catalase was estimated by the method of Beer et al (19). The enzyme activity was expressed as Units/mg protein. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability was determined by the method of Viturro et al (20). Vitamin-C was used as a standard. Nitric oxide radical inhibition activity was determined by the method of Garrat (21). Rutin was used as a standard. Superoxide anion scavenging ability was measured by the method of Nagai et al (22). Curcumin was used as a positive control. Hydroxyl radical scavenging activity was determined by method of Halliwell et al (23). Vitamin E was used as a standard. The reducing power was determined according to the method de-

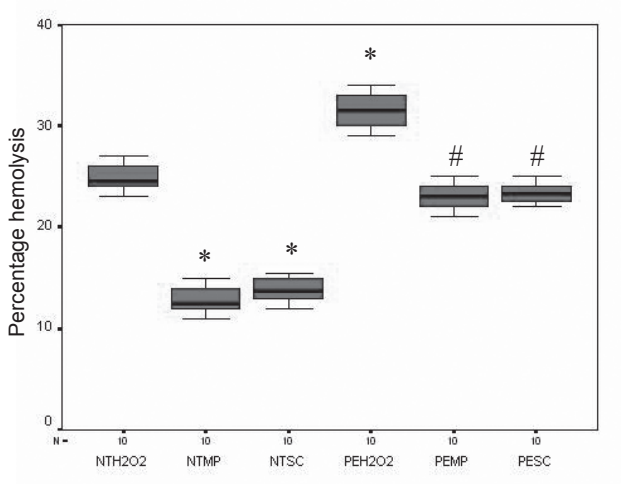

Fig. 1. H_2O_2-induced hemolysis of normotensive and preeclamptic cord blood RBC with *M. purpureus* and *S. cerevisiae.* * p<0.001 when compared with H_2O_2 induced hemolysis of normotensive cord blood RBC, # p<0.001 when compared with H_2O_2induced hemolysis of preeclamptic cord blood RBC.

veloped by method of Oyaizu (24). Butylated hydroxy toluene was used as a standard. Total antioxidant capacity of yeast extracts was evaluated by the method of Erel (25). Gallic acid was used as a standard. Total phenolic content of yeast extract was determined by method of Chandler and Dodds (26). Gallic acid was used as a standard. The phyto-components present in the aqueous extract of *M. purpureus* and *S. cerevisiae were* identified by means of qualitative analysis.

Determination of oxidative and nitrative stress

The levels of LPO were determined by the method of Ohkawa et al (27). The LPO content was expressed as nanomoles of MDA (Malondialdehyde)/mg protein. Nitrite and nitrate were estimated by the method of Yokoi et al (28). The results were expressed as µmol/mg protein.

Determination of antioxidant status in RBC after incubation

The activity of catalase was estimated by the method of Beer et al (19). The enzyme activity was expressed as Units/mg protein. Total antioxidant capacity of the yeast extracts was evaluated by the method of Erel (25).

Statistical analysis

Data were analyzed using statistical software package. Student's t-test, Two-way ANOVA was used to ascertain the significance of variations between normotensive and preeclamptic pregnant women. All data were presented as mean ± SD of 20 subject samples. Differences were considered significant at $p<0.05$, $p<0.001$. The hemolytic activity was expressed as medians for better comparative analysis. Further a Spearman rank correlation was performed to the results obtained for the anti-hemolytic activity study.

Results

Clinical Characteristics of the normotensive and preeclampsia samples analyzed are tabulated in Table 1. The normotensive and preeclamptic subjects selected for analysis had a significant alteration in the values of systolic and diastolic pressure during pregnancy while an insignificant change in those of systolic and diastolic pressure prior to pregnancy. There was also a significant decrease in the birth weight of fetus born to preeclamptic mother.

Figure 1 demonstrates the median values for H_2O_2-induced hemolysis of preeclamptic and normotensive cord blood RBC in comparison with *M. purpureus* and *S. cerevisiae*. *S. cerevisiae* reduced the hemolysis of RBC in preeclamptic condition more effectively than normotensive condition.

Tab. 2. Antioxidant status of *M. purpureus* and *S. cerevisiae*.

Parameters	Concentration (mg/ml)	Standard	M. purpureus	S. cerevisiae
DPPH.	10	25	32*	26
(% of	20	30	38*	31
inhibition)	30	36	42*	34
	40	45	47*	41
	50	50	55*	47
IC_{50}			43 mg	>50 mg
O2.-	10	17	11*	9
(% of	20	29	25*	19
inhibition)	30	38	29*	24
	40	48	42*	31
	50	54	52*	48
IC_{50}			49 mg	>50 mg
.OH	10	14	11*	10
(% of	20	16	14*	12
inhibition)	30	19	18*	14
	40	22	20*	18
	50	27	25*	20
IC_{50}			20 mg	30 mg
NO.	10	3	4	8*
(% of	20	8	6	15*
inhibition)	30	12	7	18*
	40	20	9	21*
	50	25	12	26*
IC_{50}			>50 mg	49 mg
Reducing	0.2	0.03	0.06#	0.04
activity	0.4	0.05	0.08#	0.06
(absorbance at	0.6	0.08	0.10#	0.07
700nm)	0.8	0.10	0.12#	0.09
	1.0	0.14	0.13#	0.11
IC_{50}			0.3 mg	0.6 mg
ABTS.+	20	0.5	0.48*	0.29
(mgs of	40	0.65	0.51*	0.38
equivalence	60	0.79	0.69*	0.51
of gallic acid)	80	0.98	0.96*	0.74
	100	1.25	1.17*	0.99
IC_{50}			57 mg	69 mg
Total phenolic	10	1.03	0.98*	0.74
Content	20	1.11	1.02*	0.80
(mg catechins	30	1.19	1.05*	0.86
equivalent/g)	40	1.22	1.15*	0.98
	50	1.20	1.18*	1.07

*p<0.001 when compared with the standard; #p<0.05 when compared with the standard

Tab. 3. Levels of glucose 6-phosphate dehydrogenase, lipid peroxide, nitrite and nitrate levels, total antioxidant capacity and catalase activity in cord blood RBCs of normotensive and preecla preeclamptic pregnant women with *M. purpureus* and *S. cerevisiae*.

S.No.	Parameter	Normotensive pregnant women			Preeclamptic pregnant women		
		Cord blood RBC	RBC with M. purpureus	RBC with S. cerevisiae	Cord blood RBC	RBC with M. purpureus	RBC with S. cerevisiae
1	Glucose 6 phosphate Dehydrogenase (units/1012 RBC)	526±97	556±81	523±76	278±75*	299±67	287±63
2	Lipid peroxide (mmoles/mg protein)	2.408±0.24	1.812±0.22**	2.098±0.21	2.912±0.20*	2.329±0.18**	2.614±0.16
3	NO2- (µmol/mg protein)	0.324±0.03	0.305±0.02	0.289±0.01**	0.683±0.08*	0.604±0.05	0.475±0.03**
4	NO3- (µmol/mg protein)	0.425±0.06	0.398±0.04	0.312±0.03**	0.791±0.07*	0.714±0.04	0.499±0.03**
5	TAC (mg equivalence of gallic acid)	0.532±0.04	0.689±0.03**	0.613±0.01	0.42±0.03*	0.575±0.02**	0.520±0.01
6	Catalase (Units/mg protein)	8.00±0.98	9.97±0.87**	9.07±0.85	7.14±0.87*	8.97±0.81**	8.13±0.79

* p<0.001 when compared with RBC isolated from normotensive pregnant women, ** p<0.05 when compared to cord blood RBC

M. purpureus demonstrated a highly significant DPPH, superoxide anion, hydroxyl radical scavenging activities (p<0.001) compared to *S. cerevisiae.* However, *S. cerevisiae* exhibited a highly significant nitric oxide radical scavenging activity (p<0.001), compared to *M. purpureus*. The IC_{50} values of the two yeast species are demonstrated in Table 2. The study results confirmed a significant reducing power (p<0.05) and a highly significant TAC (Total antioxidant capacity) activity (p<0.001) in extracts of *M. purpureus* when compared with those of *S. cerevisiae*. The aqueous extract of *M. purpureus* possessed higher catalase (36.67 Units / mg protein) activity compared to aqueous extract of *S. cerevisiae* (21.47 Units / mg protein). The levels of antioxidant in the aqueous extracts of *M. purpureus* and *S. cerevisiae* are shown in Table 2. The qualitative analysis demonstrated the presence of phytocomponents like flavonoids, triterpenoids, alkaloids, saponins, tannins, quinines and coumarins in the aqueous extracts of *M. purpureus* and *S. cerevisiae.*

RBCs isolated from normotensive and preeclamptic women were confirmed by analysis of RBC marker enzyme G6PDH (Glucose 6-phosphate dehydrogenase) and by microscopic analysis using Giemsa staining. There was a significant decrease in the G6PDH level in preeclamptic cord blood RBC when compared to the normotensive cord blood RBC as demonstrated in Table 3. Levels of LPO and nitrite/nitrate elevated significantly (p<0.05) in preeclamptic subjects when compared with normotensive subjects. Similarly the TAC and catalase values showed a significant (p<0.05) decrease in cord blood RBCs of preeclamptic subjects comparatively (Tab. 3). The incubation study results revealed that the activity of *M. purpureus* and *S. cerevisiae* were similar in the normotensive and preeclamptic subjects. The LPO inhibiting effect of the aqueous extract of *M. purpureus* was significantly higher than (p<0.05) that of aqueous extract of *S. cerevisiae* in both the samples. The nitrite/nitrate formation inhibiting effect of the aqueous extract of *S. cerevisiae* was significantly (p<0.05) higher than that of aqueous extract of *M. purpureus* in both the samples.

The correlation analysis of the anti-hemolytic activity in both the preeclamptic and normotensive samples treated with *M. purpureus* and *S. cerevisiae* is tabulated in Table 4. There was a significant positive correlation between the preeclamptic and normotensive samples treated with *M. purpureus*, while there was an insignificant positive correlation between the normotensive and preeclamptic sample treated with *S. cerevisiae,* suggesting that the latter yeast species functions efficiently with increasing stress.

Tab. 4. Spearman rank correlation coefficients between the normotensive and preeclamptic cord blood RBC with *M. purpureus* and *S. cerevisiae.*

Variable 1	Variable 2	r_s	p
PEMP	PESC	0.660	<0.01
	NTMP	0.614	<0.01
NTSC	NTMP	0.331	<0.05
	PESC	0.222	NS

PEMP – Preeclamptic sample treated with *M. purpureus,* PESC – Preeclamptic sample treated with *S. cerevisiae,* NTMP – Normotensive sample treated with *M. purpureus,* NTSC – Normotensive sample treated with *S. Cerevisiae,* r_s – Spearman's rho (correlation coefficient), NS – not significant

Discussion

Pregnancies complicated by HELLP syndrome are associated with increased materno-fetal mortality and morbidity (29). A significant increase in H_2O_2-induced hemolysis noted in preeclampsia indicates a deficient antioxidant status in preeclamptic cord blood RBC compared with normotensive cord blood RBC. Surface antioxidants in RBC like vitamin C can lower the H_2O_2-induced hemolysis (30). The significant positive correlation noted in normotensive and preeclamptic cord blood RBC treated with *M. purpureus* and *S. cerevisiae* suggest that both yeast species function in a similar manner to reduce the H_2O_2-induced hemolysis. The insignificant positive correlation in the normotensive and preeclamptic cord blood RBC treated with *S. cerevisiae* suggests minimal difference in the hemolytic function compared with *M. purpureus*. The percentage of hemolysis in preeclamptic sample treated with *M. purpureus* (23 %) and *S. cerevisiae* (24 %) is near to H_2O_2-induced hemolysis in normotensive sample (25 %). The

hemolysis of RBC in preeclampsia after treatment with *M. purpureus* and *S. cerevisiae* are similar to that of normotensive sample (Fig. 1), indicating that both yeast species are effective in reducing the ROS-mediated hemolysis in the preeclamptic sample.

Studies have demonstrated that oxidative stress is one of the key factors in complicating preeclampsia which results in preterm birth (31). Oxidative stress is the presence of ROS in excess of the buffering capacity of available antioxidants (32). Free radicals and other damaging ROS such as superoxide anions are involved in oxidative metabolic processes; their activation is thought to increase during preeclampsia (33). Insufficient antioxidant capacity leads to oxidative stress and subsequently the oxidative injury may occur in both the maternal and placental compartments (33). The increase in oxidative stress and nitrative stress observed in cord blood RBC in this study is in coherence with the evidence observed till date, thus suggesting their significant role in the pathophysiology of preeclampsia (34). Many studies confirm that levels of antioxidants are reduced in the sera, placentas and placental mitochondria of preeclamptic women (31, 32, 35, 36). Similarly in the present study, it was noted that the antioxidant status in preeclamptic cord blood RBC was significantly decreased compared with the normotensive sample. Administration of natural antioxidants in early pregnancy may help in preventing the complication to both mother and fetus during preeclampsia (37).

Nitric oxide-mediated nitrative stress plays an important role in the pathogenesis of preeclampsia. The increase in nitrite and nitrate levels noted in cord blood RBC in this study indirectly reflects the increase in nitrative stress during preeclampsia. NO is involved in a wide range of both physiologic and pathologic events. NO can enhance ROS toxicity due to its rapid reaction to form peroxynitrite ($ONOO^-$). Peroxynitrite is a potentially harmful ROS as it causes nitrosylation of tyrosine residues, leading to changes in protein conformation and its inactivation. The effect of elevation in peroxynitrite, and that of reduced bioavailability of NO result from enhanced production of free radicals.

The resultant placental oxidative state may contribute to the elevation of maternal blood pressure, proteinuria, platelet dysfunction as well as levels of thromboxane and prostacyclin in preeclampsia (38). *In vitro* NO inhibition study results showed that *S. cerevisiae* significantly reduced the levels of nitrite and nitrate in both samples.

Our experimental results suggest that *M. purpureus* and *S. cerevisiae* possess a wide range of antioxidant activities. The evaluation of antioxidant activity of aqueous extracts of *M. purpureus* and *S. cerevisiae* revealed a highly significant DPPH radical, superoxide anion radical, hydroxyl radical and $ABTS^+$ (2, 2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid) radical cation scavenging potentials. However, *M. purpureus* possessed a significant reducing power whereas *S. cerevisiae* demonstrated a significant nitric oxide radical scavenging activity. Both yeast species had a significant catalase activity in normotensive and preeclamptic conditions. The aqueous extract of *M. purpureus* and *S. cerevisiae* attained the IC_{50} values for different scavenging assays at varying concentration compared with standard. *M. purpureus* reached IC_{50} at a lower concentration compared to *S. cerevisiae* in scavenging free radical whereas the IC_{50} value of *S. cerevisiae* in scavenging RNS was much lower than that of *M. purpureus*. Preliminary phytochemical analysis demonstrated the presence of various biologically active constituents like flavonoids, triterpenoids, alkaloids, saponins, tannins, quinines and coumarins in the aqueous extracts of both *M. purpureus* and *S. cerevisiae.*

The efficacy of *M. purpureus* and *S. cerevisiae* extracts in quenching ROS and RNS (Reactive nitrogen species) was confirmed by the inhibition of formation of LPO and nitrite/nitrate in cord blood RBC of preeclamptic and normotensive subjects. Both extracts decreased the levels of LPO and nitrite/nitrate in normotensive and preeclamptic cord blood RBC. *M. purpureus* was more effective in inhibiting LPO formation compared to *S. cerevisiae*. In contrast, *S. cerevisiae* exhibited a significant effect in preventing the formation of nitrite and nitrate levels in cord blood RBC of both subjects. The medicinal value of *M. purpureus* in various clinical conditions has been well demonstrated, while the medicinal value of *S. cerevisiae* has seldom been demonstrated. A study by Moyad et al (39) has demonstrated the usage of Epicor, a yeast-based product against common cold and flu. The latter study is the only report analyzing the medicinal property of *S. cerevisiae.* The present study is the first of its kind to demonstrate the medicinal value of both *M. purpureus* and *S. cerevisiae* under conditions of preeclamptic pregnancy.

Conclusion

This study results conclude that anti-hemolytic and antioxidant properties of *S. cerevisiae* which is mainly considered as nutritive yeast equal those of *M. purpureus*. The research work also demonstrates for the first time that diet enriched with yeasts like *M. purpureus* and *S. cerevisiae* could serve as a natural antioxidant source and good probiotic supplement for preeclamptic patients. It protects the fetus from ROS-mediated damage by stabilizing the oxidative stress-antioxidant status imbalance as well as by inhibiting ROS-mediated RBC hemolysis under such conditions.

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