Antioxidant status

Antioxidant status was assessed by analyzing SOD, TAC and GRR in both normal and GDM placental homogenate (**Table 1)**. The level of SOD, TAC and GRR were significantly decreased by 34%, 26% and 26% in GDM placental tissue than the normal placental tissue. After incubation with tea, the levels of SOD, TAC and GRR were higher in normal placental tissue by 9%, 10%, 8% and by 10%, 12%, 13% in GDM placental tissue. The mint extracts significantly increased the level of SOD, TAC and GRR in normal placental tissue by 12%, 11%, 12% and by 17%, 16%, 17% in GDM placental tissue. When incubated with mint-tea extracts, the level of SOD, TAC and GRR increased significantly by 38%, 38%, 30% in GDM placental tissue ($p<0.001$) as compared to normal placental tissue by 13%, 18%, 14%.

Table 1: Level of SOD TAC and GRR in the normal and GDM placental tissue homogenate before and after incubation with tea, mint and mint-tea extracts

Parameters	N	NT	NM	NMT	GDM	GDMT	GDMM	GDMMT
SOD (units / mg protein)	2.892	3.165^{NS}	3.283*	3.317*	1.890$^{\$}$	2.093$^{\alpha}$	2.271$^{\&}$	2.615$^{\#}$
TAC (Trolox equivalents in mmol / L)	0.445	0.495^{NS}	0.507*	0.549*	0.230$^{\mu}$	0.261$^{\&}$	0.273$^{\&}$	0.370$^{\#}$
GRR (GSH:GSSG)	0.682	0.745^{NS}	0.769*	0.792*	0.503$^{\mu}$	0.437$^{\&}$	0.417$^{\&}$	0.718$^{\#}$

SOD (Units/mg protein), TAC (Trolox equivalents in mmol/ L), GRR (GSH:GSSG)

Normal (N) placental tissue homogenate	*GDM placental tissue homogenate*
N- without any incubation	GDM- without any incubation
NT- with tea	GDMT- with tea
NM- with mint	GDMM- with mint
NMT- with mint-tea	GDMMT- with mint-tea

$^{\$}p<0.001$, $^{\mu}p<0.01$, $^{*}p<0.05$, ^{NS} not significant; when compared with normal placental tissue homogenate without any incubation
$^{\&}p<0.05$, $^{@}p<0.01$, $^{\#}p<0.01$ when compared with GDM placental tissue homogenate without any incubation

HSP70 Expression

The expression of HSP70 was analyzed in the placental tissue homogenate of both normal and GDM pregnant women (**Fig. 5)**. The results revealed that HSP70 level was significantly increased by 45% in GDM placental tissue than normal placental tissue. Addition of tea, mint and mint-tea decreased HSP70 levels in GDM placental tissue. The level of HSP70 was decreased by incubation with tea, mint and mint-tea extract in normal placental tissue by 3%, 5% and in GDM placental tissue by 9% and by 22%, 25% and 29% respectively.

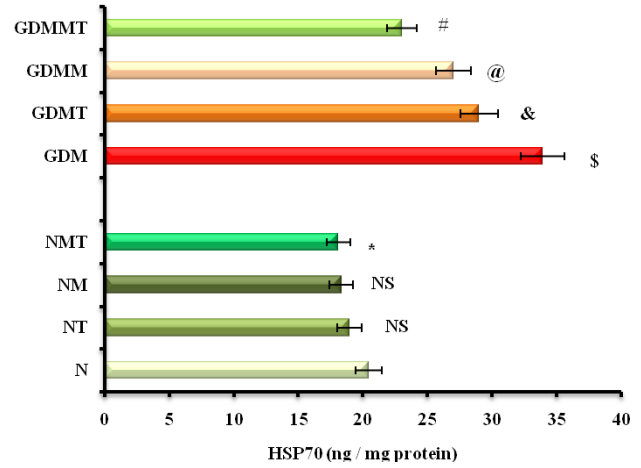

Figure 5: Level of HSP70 in the normal and GDM placental tissue homogenate before and after incubation with tea, mint and mint-tea extracts. Values are expressed as mean ± SD (for 10 samples in each group).

$^{\$}p<0.001$, $^{*}p<0.05$, ^{NS} not significant; when compared with normal placental tissue homogenate without any incubation.
$^{\&}p<0.05$, $^{@}p<0.01$, $^{\#}p<0.01$ when compared with GDM placental tissue homogenate without any incubation.

4. Discussion

One of the most prevalent complications of pregnancy is gestational diabetes mellitus, a heterogeneous disorder which is associated with both neonatal morbidity and obstetric complications (24,25). Oxidants mediated damage plays a prime role in the induction of later life complications for both mother and fetus. During oxidative stress there is an imbalance of prooxidant and antioxidant factors which can result in severe placental cell dysfunction (26). Various conventional methods were followed in the treatment of GDM. However the treatment itself turns out to be a major peril for the GDM patients. Hence the requirement of natural medicine from herbs is more, which should render the efficient antioxidant properties. Mint and tea are well known herbs with potential antioxidants and they were utilized in the present study to develop alternative antioxidant therapy for GDM pregnancy.

Free radical mediated oxidative stress has been implicated in the pathogenesis of diabetes mellitus and its complications (27,28). Elevated glucose levels and low insulin sensitivity has been suggested to be the cause of oxidative stress in diabetes which eventually leads to free radical generation ((29). It results in the production of stress markers which will further induce the intracellular peroxide production, leading to exacerbated oxidative stress. Studies suggested that increased OS products such as LPO and ADMA concentrations were induced and positively correlated with the glucose levels (30). Similarly in the present study elevated LPO, NO_2^-, HNE and ADMA were observed in placental homogenate of GDM patients. Their increased expression is believed to be largely responsible for the presence of complexities during GDM condition. Increased ADMA concentration also reflects the abnormal vascular development of GDM placenta (31). However aqueous extract of tea, mint and mint-tea reduced their level suggesting their crucial requirement during pregnancy

complications like GDM. It also depicts that it may be due to the antioxidant potency of tea, mint and mint-tea (32).

Antioxidant is a substance which significantly inhibits the oxidation of the substrate and has the potential to re-enforce our body's natural defense against free radicals and minimize damage to the body. Oxidative imbalance results in abnormal placentation and placental dysfunction. Hence the present study analyzed the antioxidant defense mechanism in GDM placenta through the assay of SOD, TAC and GRR. A significant decrease in the levels of SOD, TAC and GRR was observed in GDM placental tissue when compared to normal placental tissue which is similar to result observed by Carone (33). It is suggesting that the decreased activity may be due to its role as an antioxidant enzyme for scavenging free radicals produced in this condition. However mint, tea and mint-tea incubation shift the endogenous antioxidant to be maintain in the GDM placenta. It might be due to the antioxidant efficiency of the mentioned extracts which proficiently increase the antioxidants and diminish the stress markers.

HSP70 was quantified in the present work as it is a crucial molecular chaperones act as secondary line of defense. It is altered in response to oxidative stress to maintenance of the cell homeostasis (34). The first line of defense for oxidative stress is not sufficient to combat with the generated stress. Hence the second line defense mechanism will be activated which was reflected by the propagation of HSP70 expression, an important means of cell protection during physiological stress (35,36). Coherently, HSP70 expression was significantly increased in the GDM placenta which suggests its antiapoptotic role in GDM condition. However mint, tea and mint-tea decrease the HSP70 in GDM placental homogenate which may be due to the subsequent effect of the extracts in diminishing oxidants and restoring inbuilt antioxidants. It is depicting that HSP70 is crucial for rendering cytoprotective during pregnancy complications like GDM.

The present study investigated the antioxidant effects of mint, tea and mint-tea in GDM placenta. Both mint and tea exert the potent antioxidant activity, however mint-tea extract combination exhibits more than their sole use. It is concluded that mint, tea and mint-tea can be employed as an alternate medical care for oxidative damage during GDM condition.

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