ANTIOXIDANT STATUS OF METHANOLIC EXTRACT OF *BRASSICA RAPA CHINENSIS* LEAVES USING MAMMALIAN LIVER SLICE TECHNIQUE (*IN VIVO SIMULATED IN VITRO* MODEL)

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ABSTRACT

The influence of the methanolic extract of *Brassica rapa chinensis* (*MEBRC*) leaves on the enzymic and non-enzymic antioxidant activity in the liver tissue. The model was used an *in vitro* model which was very carefully designed to stimulate *in vivo* intraperitoneal exposure of oxidant. This study was formulated with the recommendations laid down by FRAME (Fund for the Replacement of Animals in Medical Experimentation) to minimize the use of live animals for experimenting and to develop a model system that would stimulate *in vivo* conditions. The goat liver was selected as the mammalian tissue to determine the antioxidant effect of methanolic extract in the presence and absence of the standard oxidizing compound (H₂O₂) and the dose of H₂O₂ used was the same as the level used *in vivo* studies by intraperitoneal administration (5µl/250mg tissue). The enzymic antioxidants assay such as superoxide dismutase (SOD), catalase(CAT), glutathione peroxidase(GPx), Gutathione-S-transferase (GST), Glutathione reductase (GR) and Glucose-6-phosphate dehydrogenase (G6PD). The non-enzymic antioxidants determined were total reduced glutathione (TRG/GSH), vitamin-C and vitamin-E. Lipid peroxidation (LPO) was also measured in terms of MDA formed.

KEY WORDS: Goat liver slice, Antioxidants, *Brassica rapa chinensis*, Bok choy.
INTRODUCTION
Liver is an organ of paramount importance, which plays a pivotal role in regulating various physiological processes in the body, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles. The damage to the liver caused by hepatotoxic agents is of grave consequences.\(^1\)

The free radicals can be overcome by substances called antioxidants. They may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions.\(^2\) The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Vitamin C, vitamin E, selenium, b-carotene, lycopene, lutein and other carotenoids used as supplementary antioxidants.

Oxidative stress in the cells or tissues refers to the enhanced generation of reactive oxygen species (ROS) and/or depletion in the antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants. This imbalance can ultimately result in damage to molecules, including proteins, lipids, carbohydrates and DNA. ROS would assault on important biological molecules such as DNA, protein or lipid leading to many degenerative diseases, such as cancer, Alzheimer’s, arthritis and ischemic reperfusion.\(^3\)

There are both enzymatic and non-enzymatic mechanisms that protect tissues from oxidative injuries. Antioxidant enzyme systems include superoxide dismutases (CuZnSOD and MnSOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes. One of the most important non-enzymatic endogenous antioxidants is glutathione (GSH). GSH is reported to protect cell from free oxygen radicals by a direct interaction with hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl (OH), superoxide (O\(_2^-\)) and alkoxyl (RO•) radicals.\(^4,5\) Cellular redox is maintained by a delicate balance between the cellular enzyme system that produces ROS and the antioxidant enzyme system that degrade ROS. The events that alter the balance in favor of production versus detoxification of ROS could result in excess over production of ROS thus leading to cellular injury. Antioxidant agents play an important role as part of the mechanism that protects against tissue damage caused by reactive oxygen species.\(^6,7\) Naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and barks, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress.

\(^{1,2,3,4,5,6,7}\)
Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian traditional medicine like Ayurveda, Siddha and unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases.\[8\]

The medicinal plants are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs.\[9\] The essential value of some plants has been published, but a large number of unexplored to date. There is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. Medicinal plants are of great importance to the health of individuals and communities.

Recently, natural foods and food-derived antioxidants, such as vitamins and phenolic phytochemicals, against oxidative damage are considered beneficial for human health.\[10\]

Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents.\[11\]

Phytochemicals derived from plants are excellent antioxidants. Antioxidants appear to act against diseases by raising the levels of endogenous defense, by up-regulating gene expressions of the antioxidant enzymes \[12, 13\]

The phytonutrients found in Bok choy are powerful antioxidants that are capable of strengthening in the immune system. Intake of this vegetable could reduce the risk of osteoporosis.

The present study was undertaken to investigated the antioxidant status of methanolic extract of \textit{Brassica rapa chinensis} (MEBRC) leaves using mammalian liver slice technique (\textit{in vivo} simulated \textit{in vitro} model)

**MATERIALS AND METHODS**

**Preparation of leaf extracts**

Bok choy, \textit{Brassica rapa chinensis} was obtained from local department store, Coimbatore, Tamilnadu, India.

The Fresh leaves were cleaned to remove adhering dust particles, washed under running tap water, and rinsed with distilled water. The leaves were shade dried and powdered. 15g of
dried powder was extracted in 150ml of methanol for 6 hours using a series of soxhlet extractor. The extract was filtered through Whatmann No.1 filter paper. The filtered sample was concentrated and dried under room temperature, which is denoted as methanolic extract of *Brassica rapa chinensis* (MEBRC). The extract yielded a green residue solid weighing 2.5g and was preserved in a refrigerator at 4°C.

**Preparation of mammalian liver slices:** The liver was collected freshly from local slaughter house immediately after the sacrifice of the animal. The tissue was quickly plunged into cold sterile HBSS buffer \( \text{H}_2\text{O}_2 \) concentration of 5\( \mu \text{l}/250\text{mg tissue} \).

250mg portion of the slices were used for the assay. The slices were taken in 1ml HBSS and treated with \( \text{H}_2\text{O}_2 \) (5\( \mu \text{l} \) of 30% solution), with or without 20\( \mu \text{l} \) of the leaf extract. The slices were incubated at 37°C in a water bath for 1 hour.

The goat liver slices were divided into 4 groups.

**Group I** : Control
**Group II** : \( \text{H}_2\text{O}_2 \) induced (5\( \mu \text{l}/250\text{mg tissue} \))
**Group III** : \( \text{H}_2\text{O}_2 \) induced (5\( \mu \text{l}/250\text{mg tissue} \)) + Treatment with methanolic extract of *Brassica rapa chinensis* leaves at 20\( \mu \text{l}/1\text{ml} \) of HBSS
**Group IV** : Treatment with methanolic extract of *Brassica rapa chinensis* leaves at 20\( \mu \text{l}/1\text{ml} \) of HBSS.

After the incubation period, the tissues were homogenized in the same aliquot of the HBSS buffer using a Teflon homogenizer and centrifuged to remove the debris. The supernatant was then used for the estimation of various parameters to assess the antioxidant potential. This experiment was conducted in triplicates.

The enzymic antioxidants assay such as superoxide dismutase, \cite{14} catalase, \cite{15} glutathione peroxidase, \cite{16} glutathione-s- transferase, \cite{17} glutathione reductase, \cite{18} glucose 6 phosphate dehydrogenase, \cite{19} and non- enzymic antioxidants like total reduced glutathione, \cite{20} vitamin-C \cite{21} and vitamin-E \cite{22} were determined. Lipid peroxidation, \cite{23} was also measured in terms of MDA formed.

**STATISTICAL ANALYSIS**

The data are expressed as mean ± S.D. Statistical comparison was done at significance level, \( p<0.05 \) using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.


RESULTS
The results of both enzymic and non-enzymic antioxidants and the LPO level of methanolic extract of *Brassica rapa chinensis* (MEBRC) treated, liver slices intoxicated with H$_2$O$_2$ are presented in Table 1 and 2.

A significant decrease in the levels of enzymic antioxidants SOD, CAT, GPx, GST, GR & G6PD and non-enzymic antioxidants GSH, Vitamin C & Vitamin E with an elevated levels of LPO was found in H$_2$O$_2$ (5µl/250mg tissue) intoxicated animal (group II) when compared to normal control (group I).

After the treatment with methanolic extract of *Brassica rapa chinensis* (MEBRC) leaves, a significant increase (P<0.05) in enzymic & non-enzymic antioxidants and significant decrease (P<0.05) in LPO was observed when compared to group II. Significant difference was not found in the liver slice treated with *Brassica rapa chinensis* leaves alone when compared to group I.

Table 1: Effect of methanolic extract of *Brassica rapa chinensis* on the enzymic antioxidant activity and non-enzymic antioxidant levels in goat liver.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymic antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>13.27±0.14</td>
<td>5.84±0.32</td>
<td>9.96±0.20</td>
<td>13.17±0.16</td>
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<tr>
<td>CAT</td>
<td>19.69±0.54</td>
<td>15.70±0.66</td>
<td>20.25±0.71</td>
<td>21.06±0.50</td>
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<tr>
<td>GPx</td>
<td>6.38±0.54</td>
<td>4.69±0.45</td>
<td>6.27±0.23</td>
<td>6.15±0.41</td>
</tr>
<tr>
<td>GST</td>
<td>0.44±0.2</td>
<td>0.17±0.2</td>
<td>0.25±0.2</td>
<td>0.41±0.1</td>
</tr>
<tr>
<td>GR</td>
<td>7.95±0.16</td>
<td>5.63±0.43</td>
<td>6.67±0.39</td>
<td>7.87±0.02</td>
</tr>
<tr>
<td>G6PD</td>
<td>1.68±0.11</td>
<td>1.08±0.05</td>
<td>1.43±0.06</td>
<td>1.61±0.13</td>
</tr>
<tr>
<td>Non-Enzymic antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRG</td>
<td>26.18±0.15</td>
<td>14.15±0.02</td>
<td>22.27±0.85</td>
<td>25.41±0.18</td>
</tr>
<tr>
<td>Vitamin- C</td>
<td>14.25±0.07</td>
<td>11.19±0.02</td>
<td>12.30±0.03</td>
<td>13.80±0.27</td>
</tr>
<tr>
<td>Vitamin- E</td>
<td>15.74±0.11</td>
<td>10.66±0.26</td>
<td>12.68±0.03</td>
<td>14.97±0.02</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (n =3) *-Significant at 5% (p<0.05) ns- not significant Group comparison: a) GI vs GI   b) GIII vs GII  c) GIV vs GII

Units: SOD- inhibition of 50% nitrite formation/min/ mg protein; CAT- µmoles of H$_2$O$_2$ decomposed/min/ mg protein; GPx- µmoles of glutathione consumed /min/ mg protein; GST - µmoles of CDNB-GSH conjugate formed/min/mg protein; GR - µmoles of glutathione utilized/min/ mg protein; G6PDH - 0.01 OD change /min/mg protein.
Table 2: Effect of methanolic extract of *Brassica rapa chinensis* on the levels of LPO in goat liver.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>44.53±0.91</td>
</tr>
<tr>
<td>Group II</td>
<td>86.07±0.82a</td>
</tr>
<tr>
<td>Group III</td>
<td>53.61±1.16b</td>
</tr>
<tr>
<td>Group IV</td>
<td>42.43±0.54c</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD (n =3)

*Significant at 5% (p<0.05) ns- not significant

Group comparison: a) GII vs GI  b) GIII vs GII  c) GIV vs GII

Units: LPO- µmoles of MDA formed/g tissue.

**DISCUSSION**

SOD plays a role in the suppression of oxygen free-radical formation and the decrease of nitric oxide generation. The decreased levels of GPx and SOD indicate that lipid peroxidation is one of the most prominent factors in aflatoxin toxicity and carcinogenicity [24]

SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In *P. aculeata* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

The H$_2$O$_2$ formed by SOD and other processes is scavenged by CAT that catalyzes the dismutation of H$_2$O$_2$ into water and molecular oxygen.

CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals.[25] Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx). [26]

Glutathione, a ubiquitous thiol-containing tripeptide, functions directly as an antioxidant In vivo Glutathione peroxidase, a selenium enzyme, plays a major role in regulating the
concentration of H$_2$O$_2$.$^{[27]}$ The enzyme catalyzes the breakdown of inorganic and organic peroxides and prevents lipid peroxidation and protects the cell membrane from oxidative damage.

It is a major antioxidant enzyme in curtailing the peroxidative damage in biological system. The enzyme is found in all aerobic eukaryotes and is important in the removal of H$_2$O$_2$ generated in peroxisomes by oxidases.$^{[28]}$

Glutathione-S-transferase (GST) is one of the most important detoxification enzyme systems. It defends cells against a wide variety of toxic insults from chemicals, Metabolites, and oxidative stress.$^{[29]}$ Conjugation with glutathione by GST is an important step in the metabolism and subsequent detoxification of carcinogens like polycyclic aromatic hydrocarbons, among others. GST plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione.$^{[30]}$

Glutathione reductase, also known as GSR or GR, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfydryl form GSH, Which is an important cellular antioxidant. It is commonly believed that GR exist both in cytosol and in mitochondria. The mitochondrial form was biochemically activities of GR have also been investigated in various tissues under physiologic and pathologic conditions. The glutathione reductase is concerned between all kingdoms. By generating GSH, GR indirectly participates in the protection of cells against oxidative stress and cytotoxic compounds and is deeply involved in the maintenance of the redox status of cells.$^{[31]}$

Glucose-6-phosphate dehydrogenase enzyme involves in the pentose phosphate pathway, especially important in red blood cell metabolism. It supplies reducing energy to cells by maintaining the level of the co-enzyme NADPH.$^{[32]}$ The entire antioxidant system, as well as other reductant requiring processes, relies on an adequate supply of NADPH because it is the principal intracellular reductant for al cells. G6PD is the principle source of NADPH.$^{[33, 34]}$

Non-enzymic antioxidants include vitamin C, vitamin E and glutathione. These antioxidants are present in many foods and can also be obtained through dietary supplements.

GSH is the reducing agent that recycles ascorbic acid from its oxidised to its reduced form by the enzyme dehydroascorbate reductase. It also participates in the detoxification of xenobiotics, as a substrate for the enzyme glutathione-S-transferase. GSH is also the precursor of the phytochelatins that act as heavy metal binding peptides in plants.
Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It helps to protect cells from reactive oxygen species such as free radicals and peroxides.\[^{35}\] It is a potent inhibitor of the neoplastic process, plays an important role in the endogenous anti-oxidant system. It is found particularly at high concentrations in the liver and is known to have a key function in the protective process. Glutathione is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione within cells is often used scientifically as a measure of cellular toxicity.\[^{36}\]

Vitamin C is a very effective free-radical scavenger. It acts as a chain breaking scavenger for peroxyl radicals and acts in synergy with vitamin E. Ascorbic acid serves as the chief water-soluble antioxidant protecting tissues from oxidative stress and damage caused by free radicals.

It acts as a reductant in enzymic reactions ascorbate has been shown to have an essential role in several physiological processes in plants, including, growth differentiation and metabolism. As an oxygen scavenger, ascorbic acid serves as a reducing agent. It transfers its hydrogen atoms to oxygen, making the oxygen unavailable for further reaction.

The most important function of vitamin E has been suggested to be in cell signaling (and it may not have a significant role in antioxidant metabolism).\[^{37, 38}\]

As an antioxidant, vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state.\[^{39}\] As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage. Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids.\[^{40}\]

MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissue than in normal tissues.\[^{41}\] Superoxide radical and its dismutation product H\(_2\)O\(_2\), in the presence of iron give rise to hydroxyl, starting lipid peroxidation.\[^{42}\]

**CONCLUSION**

In conclusion, the methanolic extract of *Brassica rapa chinensis* leaves showed the antioxidant activities and the level of LPO indicate the potential antioxidant effect in H\(_2\)O\(_2\)
induced free radicals in goat liver model, due to the presence of secondary metabolites like phenolic compounds such as flavonoids, and other phytochemicals.

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**REFERENCES**


