

# Impact of Combined Vitamins Supplementation in BPA Induced Swiss Albino Mice (*MUS Musculus*)

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**Abstract:** Bisphenol A BPA is an extensively used chemical found in consumer goods and food products, to which human beings are exposed. This study aims to evaluate the damaging effect of BPA in albino mice and investigate the role of combined vitamins as antioxidants against BPA - induced oxidative stress. BPA - induced albino mice were treated with combined vitamins, resulting in increased levels of enzymes such as GST, GPx, SOD, and catalase, and a decrease in the level of TBARS. These findings suggest that combined vitamins could act as antioxidants against BPA - induced oxidative stress. This study aims to evaluate the damaging effect of BPA in albino mice and investigate the potential protective role of combined vitamins as antioxidants against BPA - induced oxidative stress. Understanding the damaging effect of BPA and exploring potential protective strategies is crucial due to the widespread exposure of human beings to this chemical. This study contributes to the knowledge of BPA - induced oxidative stress and highlights the potential role of combined vitamins as antioxidants, which may help mitigate the adverse effects of BPA.

**Keywords:** BPA, Combined vitamins, Antioxidant, Albino mice

## 1. Introduction

Industrial advancement and agricultural development are inevitably linked to increase pollution and likelihood of environmental contamination by various harmful chemicals such as pesticides or industrial wastes which may further add - up in successive food chain. As per recent reports, these chemicals hold the ability to interfere with the metabolic homeostasis, which may lead to several disease morbidities (Philips *et al.*, 2018; Perrot - Applanat., 2018).

Some chemicals such as BPA, due to their micromolecular nature, can cross the blood brain barrier and could act as potential neurotoxins and may affect neuronal signaling pathways; involving reactive oxygen species; oxidative stress, calcium signaling and mainly neurotransmitters homeostasis across the nervous system (Wang *et al.*, 2019).

BPA is an important and notorious chemical, used at the industrial scale in the production of numerous commonly used products including polycarbonate plastics (Krishnan *et al.*, 1993; Biles *et al.*, 1997;), resins for coating inner lining of food cans (Brotons *et al.*, 1995), thermal paper (Biedermann *et al.*, 2010), medical devices, food containers and construction utilities, automotive industries, optical media (CDs and DVDs), electronics and dental sealants (Olea *et al.*, 1996).

Ingestion of BPA is one of the major routes of exposure which happens via eating contaminated food, drinking water and workplace exposure (EPA, 2015).

BPA being its precursor (Nargotra Sharma *et al.*, 2018). Thermal paper and carbon paper used in the cash receipts coating is an active source of BPA toxicity.

World population capacity of Bisphenol A was 1 million tons in Year 1980s, and more than 2.2 million tons in 2009. In

2003, U. S. consumption was 856, 000 tons, 72% of which used to make polycarbonate plastics and 21% going into epoxy resins. In the U. S. less than 5% of the BPA produced is used in food contact applications, but remains in the canned food and printing application such as sales receipts.

BPA-based resins are commonly used in dentistry. Due to its major applications in the production of plastic food or beverage containers and the coating of food cans, people of different ages are inevitably exposed to BPA in daily life. BPA has been detected in the human placenta (Schonfelder *et al.*, 2002), cord blood (Yi Wan *et al.*, 2010), amniotic fluid (Ikezuki *et al.*, 2002; Yamada *et al.*, 2002), fetal liver (Cao *et al.*, 2012) and breast milk (del RiscoSun *et al.*, 2004), making exposure of human neonates and infants a very real concern today.

Moreover, many research have shown that BPA induces oxidative stress in vital organs such as the liver, and kidney (Bindumol *et al.*, 2003; Chitraet *et al.*, 2003; Kabuto *et al.*, 2004; Mourad and Khadrawy, 2012).

Several studies reported the occurrence of oxidative toxicity after BPA exposure in rats and mice (Chitraet *et al.*, 2003; Gong and Han, 2006). BPA can cause liver, kidneys, brain, and other organs injury by forming Reactive oxygen species (ROS). Moreover, the study of Bindhumol *et al.*, (2003) revealed that low doses of BPA generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress in liver of rats.

Oxidative stress is an important process in which the natural balance between pro - oxidants and antioxidants is shifted toward the oxidant side to cause biological damage (Sies, 1991).

Oxidative stress affects the major cellular components like proteins, lipids and DNA. The importance of oxidative stress is commonly emphasized in the pathogenesis of various

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degenerative diseases, such as diabetes, cancer, cardiovascular disorders or neurodegenerative diseases.

Due to highly exposure of BPA which is harmful substance, we investigate its role in the induction of oxidative stress, its effect on antioxidant enzyme parameters in blood of albino mice to evaluate the antioxidant role of combined vitamins. Each of the vitamins and minerals in MVMs have a unique role to play in the body.

Vitamins and mineral supplements together maintain a delicate balance inside our body. They must cooperate to produce the better in the organisms body effect. Some dietary supplements are combined, such as combined vitamins to help us to obtain proper nutrient levels in one easy away.

## 2. Materials and Method

### 2.1 Animals

This study was conducted on 24 apparently healthy male and female Swiss Albino mice (*Mus musculus*) aged between 8 to 12 weeks with an average body weight of 32 to 40 grams. They were kept in 12 hour light and 12 hour dark cycle under the controlled temperature at 23°C degree celcius+ 2 degree celcius and relative humidity (55% +5%) conditions. Animals were acclimatized to the laboratory conditions. The animals were fed on certified pelleted rodent food, purchased from, Thansi Pets Super Stores, No.22, South Mada street, Kolathur Chennai - 600 099.

### 2.2 Experimental Design

The rats were divided into 4 groups comprising of 6 animals in each group.

Group I Control - sham operated animals (C)

Group II BPA induced (by oral) 30 days animals - Control (EC)

Group III Animals were first induced with BPA for 30 days and then from 31 to 60 days with Combined Vitamin (Vitamin A, B1, B2, B6, B12 and E) was given.

Group IV BPA was induced to animals for 1 to 3 days and immediately after 4th to 34th days BPA and Combined Vitamin was given.

The study was carried out to assess the oxidative stress, antioxidant enzymes, Glutathione S Transferase (GST), Glutathione peroxidase (Gpx), TBARS, Super oxide dismutase (SOD), Catalase (CAT), in the BPA induced animal blood. Along with the BPA induced mice were administered with Combined vitamin supplementation in powder form mixed with water for administration.

**2.3 BPA induction:** (Nimisha Balakrishnan and Sendhilvaidiv, 2016)

BPA was given to the albino mice orally (20mg/ kg body weight) for 30 days.

**2.3.1 Research Study Methods:** BPA - induced albino mice were treated with combined vitamins, and the levels of enzymes GST, GPx, SOD, catalase and TBARS were measured to assess the impact of combined vitamins

supplementation on oxidative stress. The experimental design, dosage, and duration of treatment should be provided to enhance the studys reproducibility and transparency.

**2.4 Combined Vitamins Supplementation** (Hasan *et al.*, 2011)

Mice were fed with combined vitamins (20mg/kg body weight).

**2.5 Sample Preparation:** The experimental animals were sacrificed by cervical dislocation at the end of the appropriate experimental period. The various experimental groups viz. C (Group - 1), EC (Group - 2), ET (Group - 3) BPA induced to animals for 30 days and from 31 to 60 days Combined Vitamin (Vitamin A, B1, B2, B6, B12 and E) was given. ET (Group - 4) BPA was induced to animals for 1 to 3 days and immediately after 4th to 34th days BPA and Combined Vitamins (Vitamin A, B1, B2, B6, B12 and E) was given. Immediately after the experiment the heart was punctured and the mice were dissected and heart punctured and blood sample was collected for analysis.

### 2.6 Oxidative Stress Analysis

Oxidative damage is analysed by the levels of antioxidant enzymes present in the blood samples of mice by measuring the levels of TBARS, Glutathione S Transferase (GST), Glutathione Peroxidase (GPx), Super oxide Dismutase (SOD) and Catalase (CAT).

#### 2.6.1 Glutathione S - Transferase (Habiget *al*, 1974)

Glutathione s - Transferase (GST) enzyme was assayed in all the blood samples viz C, EC, ET (Combined vitamin) 31 to 60 days and ET Combined vitamin immediately after 4<sup>th</sup> to 34<sup>th</sup> day by using the method of Habiget *al.*, 1974.

#### 2.6.2 Glutathione Peroxidase (Rotrucket *al*, 1973)

Glutathione Peroxidase (GPx) enzyme was assayed in all the blood samples of the animal viz C, EC, ET (Combined vitamin) 31 to 60 days and ET Combined vitamin immediately after 4<sup>th</sup> to 34<sup>th</sup> day by using the method of Rotrucket *al.* (1973).

#### 2.6.3 TBARS (MDA)

TBARS (MDA) was measured in all the blood samples of the animal viz. C, EC, ET Combined vitamin (31 - 60days), ET combined vitamins immediately after 4<sup>th</sup> to 34th days animals using the method of Ohkawa (1979).

#### 2.6.4 Super oxide dismutase (EC.1.151.1)

Super oxide - dismutase enzyme was assayed in all the blood samples of the animals viz. C, EC, ET Combined vitamin (31 - 60days), ET combined vitamins immediately after 4<sup>th</sup> to 34th days by using the method of Beauchamp and Fridovich (1973).

#### 2.6.5. Catalase (EC.1.11.1.6)

Catalase was measured in all the blood samples of the animals viz. C, EC, ET Combined vitamin (31 - 60days), ET combined vitamins immediately after 4<sup>th</sup> to 34th days by using the method of Chance and Machly (1955).

### 3. Statistical Analysis

The statistical analysis of the various parameters of the present study in all animal groups was carried out. Student 't' test was conducted to test the difference between two sample means by using given formula as suggested by Gupta (1978).

### 4. Results

The present study aimed to evaluate the effect of oxidative stress caused by BPA in the blood whether co-administration of combined vitamins in both prolonged (30 Days BPA, 31 – 60 days combined vitamins) and immediate (1 - 3 days BPA, 4 to 34 th day BPA and combined vitamins) form can ameliorate this oxidative damage in albino mice.

In the Experimental Control animals (EC) after administration of BPA without any treatment, The activity level of Thiobarbituric acid (TBARS) reactive substances was significantly elevated to be - 219.78 % when compared to that of Control (Table 1). The activity level of antioxidant enzymes namely Glutathione S transferase (GST), Glutathione Peroxidase (GPx), Super oxide dismutase (SOD), Catalase (CAT), are found to be reduced to 88.33%, 82.09% 81.60% and 51.03% in the BPA induced albino mice when compared to that of Control (Table - 1).

In Group – III (BPA induced to mice for 30 days and supplemented with Combined Vitamin from 31 – 60 days). The activity level of antioxidant enzymes in Group – III viz. GST, GPx, SOD, CAT, are found to be reduced to 61.15%, 53.08%, 71.89%, 20.68%, in the BPA induced albino mice when compared to that of Control (Table - 2). The TBARS value has a significant decrement 57.66% indicating lowered lipid peroxidation in the BPA induced albino mice blood supplemented with Vitamin C. (Table 2)

In Group – IV (BPA induced for 1 to 3 days followed by 4th to 34th days BPA + Combined vitamin). The results on antioxidant enzymes revealed significant improvement in the levels of GST, GPx, SOD and CAT in the blood Samples of the mice, the elevated activity levels of enzymes ranged from 21.78%, 9.44%, 59.62%, - 43.65% (Table - 2). The TBARS value has a significant decrement 27.03% indicating lowered lipid peroxidation in the BPA induced albino mice blood supplemented with Combined vitamin (Table 2).

In Group III AND Group IV the oxidative damage was found significantly reduced whereas the levels of antioxidant enzymes such as GST, GPx, SOD and CAT was observed to improve significantly.

### 5. Discussions

Vitamins and minerals play an essential role within many cellular processes including energy production and metabolism. Supplementation with of multivitamin/mineral (MVM) for  $\geq 28$  days resulted in improvements in cognition and subjective state of animals. It was demonstrated that the shifts in metabolism during cognitively demanding tasks

following MVM in females, both acutely and following 8 - week supplementation.

Dodd *et al.*, (2016) investigated the interaction between these metabolic parameters which were modulated by micronutrient interventions such as a full spectrum multivitamin/mineral (MVM) with approximately one recommended dietary allowance (RDA) of water soluble vitamins (B vitamins and vitamin C) alongside coenzyme Q10 has increased cerebral blood flow after a single dose, and a higher single dose of MVM with approximately 3 RDAs of water soluble vitamins has found to increase of fat oxidation and total energy expenditure, with the latter effect still evident after 8 weeks' of supplementation.

Sholey *et al.*, (2017) study indicated a change in the metabolic and psychological effects of MVM treatment on first day at 28 days of treatment. The acute effects of MVM supported the previous research work by Dodd *et al.*, (2016),, which demonstrated modulation of both physiological and subjective parameters.

Hasan *et al.*, (2011) studied the modulatory role of MVM on chronic unpredictable stress (CUS) and Dietary restriction (DR) that induced biochemical changes to delineate, role of micronutrient supplementation.

#### Glutathione Transferase

In Table 1 (Group – I & II), the activity level of GST in the II group was significantly decreased as compared to the control group of Albino mice in the present study. The activity level of GST was decreased to be 0.041  $\mu\text{g}$  as compared to the control, 0.3516  $\mu\text{g}$  respectively.

In Table 2 (Group – I & III), the activity level of GST in group III was significantly increased as compared to the control group of Albino mice in the present study. The activity level of GST was found to be 0.1366  $\mu\text{g}$  as compared to the control, 0.3516  $\mu\text{g}$  respectively.

In Group IV), The activity level of GST in group IV was significantly increased as compared to the control group of Albino mice in the present study. The activity level of GST was found to be 0.275  $\mu\text{g}$  as compared to the control, 0.3516  $\mu\text{g}$  respectively. The Treatment with Combine Vitamins (Vitamin A, B1, B2, B6, B12 and E) both prolonged and immediate results in significant increase in the level of GST.

#### Glutathione Peroxidase

In Table 1 (Group – I & II), The activity level of GPx in the group II was significantly decreased as compared to the control group of Albino mice in the present study. The activity level of GST was decreased to 0.0916  $\mu\text{g}$  as compared to the control, 0.5116  $\mu\text{g}$  respectively.

In Table 2 (Group – I & III) GPx Antioxidant level of the treated mice was found to be 0.24  $\mu\text{g}$  as compared to the the control value of 0.5116  $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level.

In Group – IV, the GPx Antioxidant level of the Treated mice was found to be 0.4633  $\mu\text{g}$  as compared to the the control value of 0.5116  $\mu\text{g}$ . The values were found to be

significant at  $p < 0.05$  level. The Treatment with Combine Vitamins both prolonged and immediate results in significant increase in the level of the antioxidant enzyme GPx.

### TBARS

In Table 1 (Group – I & II), The activity level of TBARS in the Experiment group was significantly increased as compared to the control group of Albino mice in the present study. The activity level of TBARS was increased to 1.636  $\mu\text{g}$  as compared to the control, 0.5116 $\mu\text{g}$  respectively.

In Table 2 (Group - I & III) The activity level of TBARS enzyme (0.2166  $\mu\text{g}$ ) was significantly decreased in the treated mice as compared to the control group of Albino mice, 0.5116  $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level in the present study.

In Group IV, the activity level of TBARS enzyme (0.3733  $\mu\text{g}$ ) was significantly decreased in the treated mice as compared to the control group of Albino mice, 0.5116  $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level in the present study. The Treatment with Combine Vitamins both prolonged and immediate significantly reduce the damage caused by Oxidative stress and results shows decrease in the level of TBARS in Group.

### Super Oxide Dismutase

In Table 1 (Group – I & II), The activity level of SOD in the Experiment group was significantly decreased as compared to the control group of Albino mice in the present study. The activity level of SOD was found to be 0.145  $\mu\text{g}$  as compared to the control, 0.7883 $\mu\text{g}$  respectively.

In table 2, (Group – I & III), The activity level of SOD in the treated group was significantly increased as compared to the control group of Albino mice in the present study. The activity level of SOD was found to be 0.2216 $\mu\text{g}$  as compared to the control, 0.7883 $\mu\text{g}$  respectively.

In (Group – I & IV), the activity level of SOD in the Treated mice was found to be 0.3183  $\mu\text{g}$  as compared to the the control value of 0.7883  $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level. The Treatment with Combine Vitamins both prolonged and immediate results in significant increase in the level of the antioxidant enzyme.

The Treatment with Combine Vitamins both prolonged and immediate results in significant increase in the level of the antioxidant enzyme SOD.

### CATALASE

In Table 1 (Group – I & II), The activity level of CAT in the Experimental group was significantly decreased as compared to the control group of Albino mice in the present study. The activity level of CAT was decreased to 0.071  $\mu\text{g}$  as compared to the control, 0.145 $\mu\text{g}$  respectively.

In Table 2, (Group – I & III) the CAT enzyme level of the treated mice was found to be 0.115 $\mu\text{g}$  as compared to the the control value of 0.145 $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level. The Treatment with Combine Vitamins both prolonged and immediate results in

significant increase in the level of the antioxidant enzyme Catalase.

In Group – I & IV, the activity level of CAT in the Treated mice was found to increased to 0.2083  $\mu\text{g}$  as compared to the the control value of 0.145  $\mu\text{g}$ . The values were found to be significant at  $p < 0.05$  level. The Treatment with Combine Vitamins (Vitamin A, B1, B2, B6, B12 and E) both prolonged and immediate results in significant increase in the level of the antioxidant enzyme Catalase.

## 6. Conclusion

The findings of this study suggest that combined vitamins supplementation has a positive impact on BPA - induced oxidative stress in albino mice. The increased levels of antioxidant enzymes GST, GPx, SOD, catalase and decreased levels of TBARS indicate the potential antioxidant role of combined vitamins against BPA - induced oxidative stress. Further research is warranted to elucidate the underlying mechanisms and explore the therapeutic potential of combined vitamins in mitigating the adverse effects of BPA.

### Conflicts of Interests

The authors declares no conflicts of interests.

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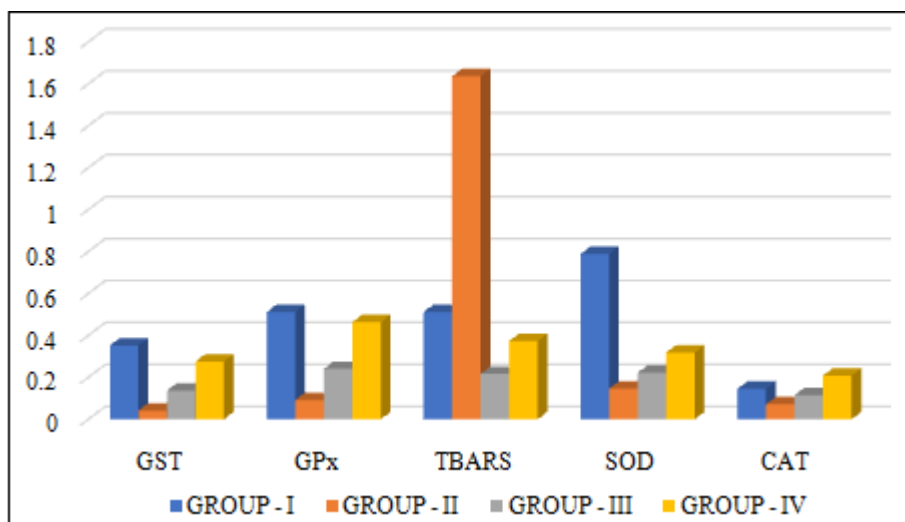
**Table 1:** Showing Parameters of antioxidant enzymes in Control (Group - I) and Experimental BPA induced Group (Group – II). Values are mean +/- SD.

S. No	Parameters	Control (Group - I)	% of Changes	Experiment (Group - II)
1	GST	0.3516 + 0.0147	88.33%	0.041 + 0.008
2	GPx	0.5116 + 0.00371	82.09 %	0.0916+ 0.018
3	TBARS	0.5116 + 0.0365	- 219.78 %	1.636+ 0.036
4	SOD	0.7883 + 0.0278	81.60 %	0.145+ 0.033
5	CAT	0.145 + 0.0137	51.03 %	0.071+ 0.013

**Table 2:** Showing Parameters of antioxidant enzymes in Control (Group - I) and BPA (1 to 30 days), combined vitamins (A, B1, B2, B6, B12 and E) and E (31 - 60 days) treated Group (Group –III) and BPA (1 to 3 days), BPA and Combined vitamins (4th – 34th days) Treated Group (Group –IV). Values are mean +/- SD.

S. No	Parameters	Control (Group I)	Experiment (Group II)	% of Changes	Experiment (Group III)	% of Changes	Experiment (Group IV)
1	GST	0.3516+ 0.0147	0.041+ 0.008	61.15%	0.1366+ 0.0103	21.78%	0.275+ 0.0273
2	GPx	0.5116+ 0.00371	0.091+ 0.0186	53.08%	0.24 + 0.0258	9.44 %	0.4633+ 0.0159
3	TBARS	0.5116+ 0.0365	1.636+ 0.036	57.66%	0.2166+ 0.0136	27.03 %	0.3733+ 0.408
4	SOD	0.7883+ 0.0278	0.145+ 0.033	71.89%	0.2216+0.0204	59.62 %	0.3183+ 0.0278
5	CAT	0.145+ 0.0137	0.071+ 0.013	20.68%	0.115+0.03594	- 43.65 %	0.2083+ 0.0116

[GST - Glutathione Transferase GPx - Glutathione peroxidase TBARS - Thiobarbituric Acid Reactive Substances SOD - Superoxide Dismutase]



**Bar Chart I:** Showing the Comparison of Mean values in GST, GPX, TBARS, SOD and CAT