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Ameliorative Role of *Lactobacillus casei* and *Bifidobacterium bifidum* in Spirotetramat induced Oxidative Stressed Wistar Rats

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ABSTRACT: Pesticides have created a severe mess throughout the agricultural world by altering various biological processes and researchers are targeting the direct their studies in order to minimize their ill effects by practicing different agents from organic background. Spirotetramat, a tetramic acid derived pesticide has been found to cause a variable toxicity among living organisms at different levels, there by causing an environmental threat to non-target organism. Keeping this challenge in the view we tried to minimize this treat with alternative intake of probiotics. In this study we have tried to find out the ameliorative role of probiotics *Lactobacillus casei* and *Bifidobacterium bifidum* in Spirotetramat induced toxicity in Wistar rats. The rats were divided in to five groups with a control group for reference. Toxicity was induced by administration of Spirotetramat at 667mg/kgbw/day for 28 days along with gavaging of probiotics (*L. casei* and *B. bifidum* 1×10^7 cfu/ml). Enzymatic activities like were estimated by prescribed protocols for estimation of oxidative stress. Lipid peroxidation (LPO), Superoxide dismutase (SOD), Catalase CAT, Glutathione peroxidase (GPx) and Glutathione (GSH) levels were estimated after sacrifice. Lipid per oxidation (LPO) was found significantly increased (302.5 % and 290.5% in Liver and Kidney respectively) (P<0.01); however in the treated groups with *L. casei* and *B. bifidum* showed a significantly (P<0.01) reduction in LPO (53.5%). Glutathione (GSH) was found to decreased significantly (P<0.01) (70.2% and 75.3% in Liver and Kidney respectively) in pesticide administrated rats as compare to untreated ones. Superoxide dismutase (SOD) levels in toxicity induced rats significantly (P < 0.01) decreased (43.5% and 49.5% in liver and kidney respectively) in comparison with untreated rats. Catalase (CAT) was also found to be decreased significantly (P < 0.01) (35.09% and 32.20% in liver and kidney respectively) that was observed in comparison to normal rats. After the treatment with *L. casei* it resulted in significant (P < 0.05, 43.08%) increase in CAT whereas in combination of both probiotics and *B. bifidum* separately it showed a significant (P < 0.01) increase in CAT. For Glutathione peroxidase (GPx) levels, a significant (P < 0.01) decrease was reported (71.20% and 67.20% in liver and kidney respectively) when compared to normal rats.

Keywords: Lactobacillus, Oxidative stress, Probiotics, Spirotetramat, Toxicity.

INTRODUCTION

Pesticides have brought a tremendous change in agricultural activities by enhancing quality and production of crops, but their toxic behavior towards organisms has always been a major worry. From past few years, global attention has increased many times in context of their toxicity and several new insecticides have been introduced by different agencies to combat this issue. The damage caused by pests is always in focus so that new pesticides with improved action can be formed with least toxicity. Spirotetramat, a potential tetramic acid derived pesticide acts by inhibiting acetyl CoA carboxylase in lipid biosynthesis in insects (Gutboard *et al*., 2020). It has been reported that spirotetramat can cause loss in weight, damage in liver and testes in rats. It also affects non target organisms and cause environmental imbalance (Liu, 2011). It has also found to be a skin irritant (Ye, 2011). It also increases the activity of acid phosphatase in rats but hinders those of alkaline phosphatase and carboxyl esterase in vivo. It has also adverse effects on aquatic fauna like trouts and carps (Wu *et al*., 2012). Yin *et al.* (2014) reported that sub-lethal doses of spirotetramat cause oxidative stress

and lipid peroxidation in toad (*Bufo bufogargarizans*) tadpoles. Being a soil contaminant, it also causes potential biochemical and genetic toxicity in earthworms (Zhang *et al.,* 2015).

Microbial dietary supplements have been found to be most effective to bring up the human health by implementation through gut in humans (Reid *et al.,* 2013; Guarner and Malagelada 2003). By acting as a potential modulator of microbes in intestinal tract. Probiotics, like *L. casei* and *B. bifidum*, both are Gram positive non sporing bacteria know to possess various health benefits. *L. casei* has been also found to be anti-inflammatory and boosting of immune system (Zhou *et al.,* 2021). It has been also reported for reduction of oxidative stress in humans by increasing antioxidant activity (Kleniewsaka and Pwaliczak 2017) and Recovered oxidative stress in rats fed with high-fat diet (Lasker *et al.,* 2019). A suppression of oxidative stress has been reported in food containing probiotics rich in *L. casei* by clamping down the $CD4^+$ T-Cells (Villarini *et al.,* 2008). *B. bifidum* metabiotics have also been found to modulate antioxidant activity and inflammatory responses in Wistar rats (Collin *et al.,* 1998). In terms of Immunosuppression and antioxidant activities, probiotics are known to produce proinflammatory molecules (So *et al.,* 2008). With profound proof of antioxidant activities of probiotics, this work was planned to reveal the antioxidant stress caused by Spirotetramat and its reduction by probiotics in Wistar rats.

METHODOLOGY

Bacterial Strains: The bacterial strains *L. casei* and *B. bifidum* were obtained from Department of Zoology, S.H.G. College and were revived using MRS (de Mann Rogosa Sharpe) agar medium.

Animals: Male Wistar rats weighing about 200-220g were purchased from Pinnacle Biomedical Research Institute wide institutional ethical committee guidelines with registration number 1824/PO/Rc/S/15/CPSCEA.

Chemicals: All the chemicals used in this study were purchased from reputed companies with a grade of high purity. The Spirotetramat was purchased from Bayer India. Various Kits were used for detection of oxidative stress parameters from different companies.

Dosing Bacterial Stock Preparation: Under anaerobic condition at 37°C for 48 hours, a culture of *L. casei* and *B. bifidum* was prepared using MRS broth. For 1 ml of distilled water a loopful of this culture was added to make the final suspension up to 10ml. A number of 1/10 dilutions were prepared From N1 to N6. From N6 100 Microliter was inoculated on MRS agar medium. A number of colonies were obtained after revival. From such colonies a healthy colony was isolated and added to 1ml distilled water to obtain a 1×10^7cfu/ml after calculation of last dilute concentration.

Stress Induction: For sub-acute toxicity, a single dose of Spirotetramat of about 667mg/kgbw/day equal to 10000ppm dissolved in water was administrated orally for 4 weeks (Young, 2006).

Dosage: Soon after probiotics were administrated orally by gavaging in rats for 4 weeks alone and in combination as per experimental protocol.

Experimental Setup: The experimental setup including five groups *viz.*, A, B, C, D and E with at least six rats of equal weight in each group and were framed as per given plan below:

Group	Remarks
A	Control
B	Toxicity Control
C	B1 Treated
D	B2 Treated
E	B1+B2 Treated

B1= *L. casei* B2= *B. bifidum*

Under mild Ether anesthesia, the rats were sacrificed after 4 weeks. Tubes carrying EDTA (Ethylene diamine tetra acetic acid) as blood anticoagulant were filled with collected blood samples from punctured heart using disposable syringes. Samples were kept as such for 30 minutes at 20°C and then centrifuged at 3000rpm for 15 minutes to isolate the plasma for biochemical estimations. After 20 min of time gap at room temperature, samples of blood were centrifuged at 3000 rpm for 10 min to separate the plasma. For one week the collected plasma samples were kept in a deep freezer until analyzed within 1 week for the biochemical estimations. Liver and Kidney were removed and washed with cold saline before homogenization. About 1 g of liver tissue and 0.5 g of Kidney tissue were homogenized separately in 2 mL of phosphate buffer saline solution 1:2 (at pH= 7.4, w/v; 1 g tissue 2 mL TBS). For determination of enzyme activities from supernatant, homogenates were centrifuged at 20,000 g for 8 min at 4°C.

Assessment of Oxidative stress: Several prescribed procedures for estimation of oxidative stress were used. For lipid peroxidation (LPO) was carried out by a method given by Ohkawa *et al.,* (1979), Superoxide dismutase (SOD) by Kakkar *et al.* (1984), Catalase CAT by Sinha (1972), Glutathione peroxidase (GPx) by Rotruck *et al.* (1973); Glutathione (GSH) by Habig *et al.* (1974)

Statistical Methods: Graph Pad InStat software was used for various calculations. According to mean of observed values results were calculated as a mean of standard error. ANOVA (one way) was used to calculate variance and comparison was carried out by Dunnet's test.

RESULTS

Oxidative stress in Liver and Kidney:

a) Lipid per oxidation (LPO). A significant increase in LPO (302.5 % and 290.5% in Liver and Kidney respectively) (P<0.01) was observed in rats administrated with pesticide as compared to control. However, the treated groups with *L. casei* and *B. bifidum* showed a significantly (P<0.01) reduction in LPO (53.5%). The group treated with *L. casei* revealed a significant (P<0.05) reduction in LPO (35.5%) and that of treated with *B. bifidum* reduced LPO significantly (P<0.01) to 47.2% in liver tissue. In kidney cells the results were similar but a little bit higher than liver tissue with a significantly (P<0.01) reduction of LPO (36.3% and 48.5% respectively) when treated with *L. casei* and *B. bifidum.*

b) Glutathione (GSH). A significant (P<0.01) decrease in concentration of GSH (70.2% and 75.3% in Liver and Kidney respectively) was reported in pesticide administrated rats as compare to untreated ones. Dosage with *L. casei*, *B. bifidum*, and both revealed significant (P < 0.01) increase in the level of GSH in the Liver (98.5%, 142.4%, and 165.4%, respectively) and kidney (95.5%, 112.4%, and 118.4%, respectively) in spirotetramat induced toxicity in rats as compared to normal control.

c) Superoxide dismutase (SOD). SOD levels in toxicity induced rats significantly (P < 0.01) decreased (43.5% and 49.5% in liver and kidney respectively) in comparison with untreated rats. Administration of *L. casei*, *B. bifidum*, and both revealed significant (P < 0.01) increase in the level of SOD in the Liver (49.5%, 46.2%, and 58.4%, respectively) and kidney (54.2%, 60.4%, and 62.4%, respectively) in spirotetramat induced toxicity in rats as compared to normal control.

d) Catalase (CAT). The concentration of CAT was found to be decreased significantly (P < 0.01) (35.09% and 32.20% in liver and kidney respectively) that was observed in comparison to normal rats. After the treatment with *L. casei* it resulted in significant (P < 0.05, 43.08%) increase in CAT whereas in combination of both probiotics and *B. bifidum* separately it showed a significant (P < 0.01) increase in CAT in spirotetramat induced rats in their tissue (57% and 51.52% in liver and 53.20% and 48.40% in kidney respectively) when compared to control.

e) Glutathione peroxidase (GPx). GPx levels were reported to be decreased significant (P < 0.01) (71.20% and 67.20% in liver and kidney respectively) when compared to normal rats. With the oral gavaging of *L. casei*, *B. bifidum*, and combination of both showed significant (P < 0.01) increase in the pancreatic tissue (58.50%, 70.20%, and 95.20%, in liver and 55.50%, 68.20%, and 85.20%, in kidney respectively) as compared to control.

Table 1: Role of Probiotics (*Lactobacillus casei, Bifidobacterium bifidum*) on oxidative stress of Liver tissue in Wistar rats.

Parameters	Control	Toxicity Control	B1 Treated	B2 Treated	B1+B2 Treated
LPO[#]	1.10±0.05	3.93±0.08^{z}	3.05±0.09^{*,z}	2.30±0.09^{**,y}	2.10±0.08^{**,x}
GSH[##]	6.80±0.10	2.50±0.15^{z}	5.10±0.20^{**,z}	5.35±0.8^{**,y}	6.10±0.55^{**,x}
SOD [###]	13.20±0.05	7.50±0.16^{z}	11.10±0.08^{**,z}	10.90±0.08^{**,z}	12.10±0.12^{**,z}
CAT[###]	30.9±1.05	20.9±0.35^{z}	25.05±2.50^{*,z}	26.50±2.2^{**,z}	29.10±01.05^{**,y}
GPx [###]	0.89±0.04	0.35±0.06^{z}	0.52±0.06^{**,z}	0.58±0.04^{**,z}	0.70±0.04^{**,z}

#) (nmol MDA/h/g) tissue; ##) (µmol/g) tissue; ###) (µmol/min/mg) protein

B1 Treated = Treated with *L. casei*, B2= Treated with *B. bifidum,* B1 + B2 = Combination.

Mean (X) ±SEM (n=*no of rats*=6 rats per group). $^{x}P>0.05$, $^{y}P<0.05$, $^{z}P<0.01$ compared to control, $^{*}P<0.05$, $^{**}P<0.01$ compared to Toxicity control.

SEM=Standard error of mean, *L. casei* (*Lactobacillus casei*) *B. bifidum* (*Bifidobacterium bifidum*), LPO-Lipid peroxidation, MDA-Malondialdehyde, GPx-Glutathione peroxidase, GSH-Glutathione. SOD-Superoxide dismutase, CAT-Catalase.

Table 2: Role of Probiotics (*Lactobacillus casei, Bifidobacterium bifidum*) on oxidative stress of kidney tissue in Wistar rats.

Parameters	Control	Toxicity Control	B1 Treated	B2 Treated	B1+B2 Treated
LPO[#]	1.05±0.05	4.83±0.06^{z}	4.05±0.06^{*,z}	2.80±0.09^{**,y}	2.30±0.06^{**,x}
GSH[##]	6.50±0.10	2.80±0.12^{z}	5.20±0.20^{**,z}	5.40±0.8^{**,y}	5.90±0.55^{**,x}
SOD [###]	12.80±0.05	7.90±0.12^{z}	10.08±0.08^{**,z}	11.60±0.08^{**,z}	11.90±0.12^{**,z}
CAT[###]	30.2±1.05	22.5±0.30^{z}	24.02±2.40^{*,z}	25.50±2.2^{**,z}	28.10±01.05^{**,y}
GPx [###]	0.79±0.06	0.42±0.04^{z}	0.48±0.04^{**,z}	0.52±0.04^{**,z}	0.65±0.06^{**,z}

#) (nmol MDA/h/g) tissue; ##) (µmol/g) tissue; ###) (µmol/min/mg) protein

B1 Treated = Treated with *L. casei*, B2= Treated with *B. bifidum,* B1 + B2= Combination.

Mean (X) ±SEM (n=*no of rats*=6 rats per group). $^{x}P>0.05$, $^{y}P<0.05$, $^{z}P<0.01$ compared to control, $^{*}P<0.05$, $^{**}P<0.01$ compared to Toxicity control.

SEM=Standard error of mean, *L. casei* (*Lactobacillus casei*) *B. bifidum* (*Bifidobacterium bifidum*)

LPO-Lipid peroxidation, MDA-Malondialdehyde, GPx-Glutathione peroxidase, GSH-Glutathione. SOD-Superoxide dismutase, CAT-Catalase.

DISCUSSION

The role of probiotics related to their favorable effects has been demonstrated clinically (Mengheri, 2008). *L. acidophilus* and *L. casei* has also been reported to fight against various intolerances like dyslipidemia and hyperinsulinemia in the body of rats (Yadav *et al.,* 2007). Pesticides have been investigated to induce oxidative stress by production of free radicals which can accumulate in living cells and can disrupt the biological activity of biomolecules or can modify their chemical behavior (Zepeda *et al.,* 2017; Mostafalou *et al.,* 2013). The generated free radicals usually cause oxidative deterioration of lipids *viz.* LPO (Oberley, 1988). For present study the level of LPO was found to be enhanced in both liver and kidney after exposure to Spirotetramat which reflects that generation of free radicals might be responsible for this elevation. After administration of probiotics the level of LPO was found to be significantly decreased in treated rats. This reflects that probiotics have an ameliorative role in decreasing LPO. For Cellular protection reduced Glutathione (GSH) acts against reactive oxygen species (ROS) (Yadav *et al.,* 2008) and acts as a co-substrate (Lu, 2009). Reduction in GSH levels in present study has been reported due to ill effect of Spirotetramat. Enhance in GSH production due to probiotics may be due to decrease in stress and biosynthesis of more GSH. Free radical production decreases the doings of GPx (Anuradha and Selvam 1993). Present study reveals the significant increase in GPx doings. The suppressed production of free radicals due to administration of probiotics has enhanced the production of GPx and its activity. SOD converts superoxide anions in to hydrogen peroxide and water by dis-mutation of superoxide radicals (Freeman and Crapo 1998). Administration of probiotics showed significant increase in SOD. Likewise CAT levels have also been reported to be increased significantly due to probiotic administration.

CONCLUSIONS

By estimating the relative oxidative stress among different groups of Wistar rats, it is finally concluded that probiotics have a promising role in control of organ toxicity and hence can be used as an alternative therapy to minimize the relative ill effects Spirotetramat.

FUTURE SCOPE

Being a newly introduced pesticide in the field of agriculture the alternative use of probiotics can minimize the ill effects on non-target organism hence degradation of biodiversity and maintenance of ecological balance can be minimized.

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Conflict of Interest: None.

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