ABSTRACT

Objective: The aim was to evaluate the effect of the polyphenolic fraction of *Desmostachya bipinnata* Stapf (PFDB) (Poaceae) on tamoxifen (TAM)-induced liver damage in female Sprague-Dawley rats.

Materials and Methods: The roots of *Desmostachya bipinnata* were extracted in 70% methanol, and the polyphenolic fraction was isolated. Protection of BRL3A cells against ethanol-induced damage was determined by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. Hepatotoxicity was induced in rats by oral administration of TAM (45 mg/kg/day) for 21 days. The PFDB was administered to experimental animals at two selected doses (100 and 200 mg/kg/day) during the treatment. The serum levels of various biochemical parameters and the antioxidant enzymes were examined by standard procedures.

Results: A dose-dependent increase in percentage viability was observed when ethanol-exposed BRL3A cells were treated with PFDB. Both the treatment groups upon pretreatment with PFDB exhibited a significant (*P* ≤ 0.05) protective effect by lowering serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, triglycerides, cholesterol, urea, uric acid, bilirubin and creatinin levels and improving protein level in serum in dose-dependent manner, which was comparable to that of silymarin group. In addition, PFDB prevented elevation of reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase in the TAM-intoxicated rats in concentration-dependent manner and significantly (*P* < 0.05) reduced the lipid peroxidation in the liver tissue. The biochemical observations were supplemented with histopathological reports, which showed the attenuation of hepatocellular necrosis.

Conclusions: The results of this study strongly indicate that the polyphenolic fraction of the plant roots has a potent hepatoprotective action against TAM-induced hepatic damage in rats.

KEY WORDS: Biochemical, *Desmostachya bipinnata* stapf, hepatotoxicity, histopathological...
radicals, thereby initiating the process of LPX. Furthermore, TAM has been shown to potentiate nitrous oxide production in breast cancer patients through enhancement of nitric oxide synthase II expression.\textsuperscript{[14]}

Due to lack of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders. Hence, it is essential to unfold the scientific basis of the antihapatotoxic nature of the traditionally useful herbal drugs. It is reported that hepatoprotective and antioxidant activity by scavenging free radicals are probably due to phenolic/flavonoid compounds present in the plant.\textsuperscript{[5]} \textit{Desmostachya bipinnata} Stapf (family: Poaceae), a perennial grass, is commonly known as “Sacificial Grass” in English and “Darbha” in Sanskrit. It pacifies pitta, asthma. In Indian traditional medicine, it is used as diuretic and in diarrhoea, dysentery, menorrhagia, jaundice, skin diseases, burning sensation and excessive in a humidified atmosphere of 5% CO\textsubscript{2}. The cells were then exposed to toxicant (medium containing 100 mM ethanol) along with/without a polyphenolic fraction of \textit{Desmostachya bipinnata} (PFDB). Concentrations of PFDB >650 μg/ml and that of standard drug Silymarin >250 μg/ml were found to be toxic to the cells; hence, concentrations in the range of 125–500 μg/ml were used for PFDB and 250 μg/ml were used for the standard Silymarin. At the end of the period, cytotoxicity was assessed by estimating the viability of the BRL3A cells by the MTT reduction assay. After 24 h incubation, the test solution from each well was removed by aspiration and replaced with 50 μl of MTT. The plates were gently shaken and incubated for 3 h at 37°C in a humidified 5% CO\textsubscript{2} atmosphere. The supernatant was removed, and 100 μl propanol was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a microplate reader at 540 nm.\textsuperscript{[12]}

**In vivo Hepatoprotective Activity on Tamoxifen-Induced Hepatotoxicity**

Female Sprague-Dawley rats (150–200 g) were used for the study. The experimental protocol was approved by the institutional animal ethical committee at KMCH College of Pharmacy, Coimbatore. The study was performed in accordance with CPCSEA guidelines. To establish the safety of the drug, acute toxicity study was carried out as per OECD–123 guidelines. It was observed that all animals tolerated mass test doses and showed no ill effects at LD50 >2000 mg/kg. Dose of PFDB was fixed at 100 and 200 mg/kg, whereas for standard silymarin at 45 mg/kg. The animals were divided into five groups of six animals in each group. Group I served as control received normal saline and was not treated with the toxicant. Group II served as TAM control. Group III received silymarin (100 mg/kg body weight). Group IV and V received PFDB (100 and 200 mg/kg body weight). The animals received these treatments by oral route for 21 days. Except Group I, all other groups received TAM (45 mg/kg body weight) p.o. form day 10–21. On 22\textsuperscript{nd} day blood was obtained from the animals by puncturing retro-orbital plexus and allowed to clot at room temperature. Serum was separated by centrifugation and utilized for estimation of biochemical parameters including serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), cholesterol, triglycerides (TG), urea, CR, uric acid and bilirubin.\textsuperscript{[13]} The livers were excised and homogenate was processed for the estimation of LPX. A part of homogenate after precipitating proteins with Trichloroacetic acid was used for estimation of glutathione. The rest of the homogenate was centrifuged and the supernatant was used for estimation of superoxide dismutase (SOD) and catalase (CAT) activities.

**Histopathological Examination**

The Liver was removed, fixed overnight in 10% buffered formalin and embedded in paraffin. 4–6 μm thick sections were made, dewaxed in xylene, rehydrated in graded alcohol.
series and washed with distilled water for 2 min. Subsequently, the sections were stained with hematoxylin for 5 min at room temperature. After 15 min, the sections were counterstained with eosin for 2 min, dehydrated in graded alcohol series, washed with xylene and observed under the microscope at ×10 and × 40 magnifications.

**Statistical Analysis**

The statistical analysis was performed using one-way analysis of variance. The values are expressed as mean ± standard deviation. Comparison of mean values was performed by Duncan’s Multiple Range Test (DMRT) using SPSS software (San Diego, USA). \( P < 0.05 \) was considered as significant.

**Results**

**In vitro Hepatoprotective Effect on BRL3A Cells**

The effects of PFDB on BRL3A cells intoxicated with ethanol are reported in Table 1. The cells exposed with ethanol showed a percentage viability of 10.302%, while the cells which were pretreated with PFDB showed a dose-dependent increase in percentage viability and the results are highly significant \( (P < 0.001, \text{ when compared to ethanol intoxicated cells}) \). Maximum protection to the cells was offered upon pretreatment with 500 \( \mu \)g/ml PFDB and was found to be 67.0035%.

**In vivo Hepatoprotective Activity**

In liver function test parameters, a remarkable elevation was observed in serum SGOT, SGPT, ALP, TG, cholesterol, urea, uric acid, bilirubin and creatinin levels in TAM-intoxicated rats. This may be due to extensive hepatic damage caused during TAM treatment. Pretreatment of rats with PFDB effectively protected them against TAM-induced hepatotoxicity, which is evidenced by decreased levels of these parameters in serum [Table 2]. The protective effect was found to be dose-dependent and comparable to standard silymarin treated rats.

The decrease in antioxidant defence systems of TAM-intoxicated rats render them more susceptible to hepatotoxicity. As shown in Table 3, the levels of antioxidant enzyme activities in liver homogenates (reduced glutathione \([\text{GSH}]\), glutathione peroxidase \([\text{GPX}]\), SOD and \([\text{CAT}]\) were significantly improved upon pretreatment of TAM-intoxicated rats with PFDB which inhibited TAM-induced hepatic injury and thereby the level of oxidative stress due to decreased LPX and enhanced antioxidant enzyme activities.

The histopathological studies revealed an extensive hepatic damage in case of TAM group. Silymarin and both the test groups showed a protective effect by decreasing the extent of centrilobular necrosis, feathery degeneration and inflammation when compared to TAM group [Figure 1].

**Discussion**

**In vitro** cell viability and hepatoprotective activity of PFDB was assessed on BRL3A cell lines upon alcohol-induced hepatotoxicity by MTT assay method. The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity, which in turn may be interpreted as a measure of viability and/or cell number. The nontoxic concentrations of PFDB tested against ethanol-induced toxicity in BRL3A offered dose-dependent protection to cells.

Tamoxifen citrate is an anti-estrogenic drug widely used for the treatment of breast cancer. It was reported that TAM in toxic doses lead to oxidative liver damage as it has been elucidated to be a hepatocarcinogen in rats.\(^{14-16}\) It may be more toxic to the liver because it has a much higher affinity for hepatic tissue than for any other tissues.\(^{17}\) It was found to produce five DNA adducts in rat liver that appeared to be responsible for carcinogenesis.\(^{18}\)

The extent of hepatic damage by TAM-intoxication was assessed by the level of released cytoplasmic transaminases (\([\text{SGOT}, \text{SGPT}]\) and phosphatase in circulation. The rise in the serum levels of aspartate aminotransferase, alanine transaminase and ALP has been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage.\(^{19}\) The results of this study demonstrate that pretreatment of rats with PFDB effectively protected them against TAM-induced hepatotoxicity. This has been evidenced by decreased serum aminotransferase activity. A remarkable elevation in serum TG, cholesterol, urea, uric acid, bilirubin, creatinin and lipid peroxidase levels was observed in TAM-intoxicated rats, whereas the level of total proteins was reduced due to the TAM-induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum, which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of TG leading to fatty liver. Inhibition of bile acids synthesis from cholesterol leading to increase in cholesterol levels was also resulted due to TAM-intoxication. Suppression of cholesterol levels by PFDB suggests the bile acids synthesis inhibition was reversed. The decrease in antioxidant defence systems of TAM-intoxicated rats render them more susceptible to hepatotoxicity. The levels of antioxidant enzyme activities in liver homogenates (\([\text{GSH}, \text{GPX}, \text{SOD} \text{and} \text{CAT}]\) were significantly improved upon pretreatment of TAM-intoxicated rats with PFDB which inhibited TAM-induced hepatic injury and thereby the level of oxidative stress as it can decrease LPX and enhance antioxidant enzyme activities [Table 3], whereas the level of TBARS were significantly decreased comparable to TAM-intoxicated group. The increase in GPX and SOD activities after PFDB administration to TAM-intoxicated rats, was attributed to induction or mutually protective interactions especially SOD, which is present at low level only but highly

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**Table 1:**

| Treatment               | Concentration (μg/mL) | Percentage viability |
|-------------------------|-----------------------|----------------------|
| Control                 | -                     | 100                  |
| Ethanol                 | -                     | 10.302±2.601*        |
| Ethanol+silymarin       | 250                   | 72.946±3.377**       |
| Ethanol+PFDB            | 500                   | 67.035±4.289**       |
|                         | 250                   | 32.276±2.235**       |
|                         | 125                   | 12.924±3.375**       |

Values are expressed as mean±SD (n=6). *\( P<0.05 \) when compared to control, \**P<0.01 \) when compared to TAM-treated group; TAM=Tamoxifen, PFDB=Polyphenolic fraction of Desmostchya bipinnata, SD=Standard deviation.
The destruction and degradation of phospholipids hydroperoxides are carried out by GPX and this suggested being a pathway of cytoprotection against the deleterious effects of phospholipids hydroperoxides.

Table 2:
Effect of PFDB on biochemical parameters in TAM-intoxicated female Sprague Dawley rats

| Biochemical parameters | Control | TAM (45 mg/kg) | TAM+silymarin (100 mg/kg) | TAM+PFDB (100 mg/kg) | TAM+PFDB (200 mg/kg) |
|------------------------|---------|----------------|---------------------------|----------------------|----------------------|
| SGOT (U/L)             | 36±4.33 | 124±9.44       | 40.5±3.62**               | 73.5±3.02**          | 60.5±1.87**          |
| SGPT (U/L)             | 39±1.79 | 100±8.51*      | 46±2.37***               | 65±4.0**             | 53±6.0±5.006**       |
| ALP (U/L)              | 71.7±4.68 | 155.75±5.04* | 92.5±4.34***             | 123.35±6.88**        | 53.67±5.006**        |
| TG (mg/dL)             | 33.1±4.23 | 96.75±12.68*  | 41±1.03.006**            | 64.7±4.32**          | 52.21±5.32**         |
| Cholesterol (mg/dL)    | 31.6±5.69 | 63.6±4.27*    | 39.05±3.26***            | 50.75±3.67**         | 42.58±2.39**         |
| Urea (mg/dL)           | 32.3±1.79 | 66±2.39*      | 34.5±0.34***             | 48.92±1.92**         | 38.21±2.5**          |
| Bilirubin (mg/dl)      | 4.8±0.36 | 7.7±0.37*     | 5.35±0.24***             | 6.45±0.23**          | 5.9±0.179**          |
| Creatinin (mg/dl)      | 0.5±0.14 | 1.6±0.14*     | 0.6±0.15***              | 1.05±0.19**          | 0.683±0.15**         |
| Protein (g/dL)         | 6.785±0.48 | 3.475±0.33*   | 5.828±0.44**             | 4.725±0.26**         | 5.257±0.26**         |

Values are expressed as mean±SD (n=6). #P<0.05 significantly different from the corresponding values of the control, **P<0.01, ***P<0.001 significantly different from the corresponding values of the TAM-treated group. TAM=Tamoxifen, PFDB=Polyphenolic fraction of Desmostachya bipinnata, SD=Standard deviation, TG=Triglycerides, ALP=Alkaline phosphatase, SGPT=Serum glutamic pyruvic transaminase, SGOT=Serum glutamate oxaloacetate transaminase

Table 3:
Effect of PFDB on antioxidant enzyme levels in TAM-intoxicated female Sprague Dawley rats

| Antioxid enzymes         | Control | TAM (45 mg/kg) | TAM+silymarin (100 mg/kg) | TAM+PFDB (100 mg/kg) | TAM+PFDB (200 mg/kg) |
|--------------------------|---------|----------------|---------------------------|----------------------|----------------------|
| LPX (moles MDA/mg protein) | 0.105±0.023 | 0.158±0.031* | 0.104±0.015**             | 0.128±0.024**        | 0.113±0.021**        |
| GSH (mmole Glu/mm/mg protein) | 0.773±0.037 | 0.259±0.042* | 0.741±0.025***            | 0.409±0.040**        | 0.621±0.022**        |
| GPX (mmole Glu/mm/mg protein) | 0.841±0.042 | 0.269±0.0649* | 0.773±0.061***            | 0.587±0.036**        | 0.760±0.07**          |
| SOD (U/mg protein)       | 29.3±1.618 | 15.416±0.934* | 28.466±1.968**            | 21.42±1.003**        | 26.716±1.665**        |
| CAT (mmole H2O2/mm/mg protein) | 5.630±0.217 | 3.380±0.273* | 4.582±0.124***            | 3.592±0.173          | 4.264±0.235**         |

Values are expressed as mean±SD (n=6). #P<0.05 significantly different from the corresponding values of the control, **P<0.01, ***P<0.001 significantly different from the corresponding values of the TAM-treated group. TAM=Tamoxifen, PFDB=Polyphenolic fraction of Desmostachya bipinnata, SD=Standard deviation

Figure 1: Effects of polyphenolic fraction of Desmostachya bipinnata on Tamoxifen-induced liver damage in rats-histopathology (stained with hematoxylin and eosin): (a) Normal group, normal cellular architecture with distinct hepatic cells (10×). (b) TAM-intoxicated group, feathery degeneration with necrosis and vacuolation (40×). (c) Silymarin (100 mg/kg) + Tamoxifen-treated group, less necrosis and vacuolation (40×). (d) PFDB (100 mg/kg) + Tamoxifen-treated group (40×). (e) PFDB (200 mg/kg) + Tamoxifen-treated group, increased number of hepatocytes with normal nucleus (40×)
The decreased GPX level of TAM-intoxicated rats leads to an increase of toxic level to the cells. The decreased activities of GPX and SOD in liver homogenate of TAM-intoxicated rats may be due to oxidative stress induced inactivation and/or exhaustion. Furthermore, it was reported that, the decreased GPX activity leads to H$_2$O$_2$ accumulation in the liver which in turn inactivates SOD. The detoxification of different drugs and xenobiotics in the liver produce GSH. GSH plays a common role in cellular resistance to oxidative damage as a free radical scavenger as protein-bound glutathione and by the generation of ascorbate or tocopherol in the liver. The decreased hepatic GSH in TAM-intoxicated rats could be as a result of hexose monophosphate shunt impairment due to TAM-intoxication and thereby NADPH availability is reduced and the ability to recycle GSSG to GSH is decreased. By blocking oxidative damage through LPA and protein oxidation, PFDB prevented the loss of membrane permeability and dysfunction of cellular proteins and decreased the endogenous level of hydroxyl radical and GSH. The liver sections of the rats treated with normal saline showed normal hepatic architecture, whereas that of TAM-treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, feathery degeneration, inflammation, and apoptosis. In case of rats treated with silymarin and PFDB (100 mg/kg and 200 mg/kg) a normal hepatic architecture was seen with only moderate accumulation of fatty lobules and mild degree of cell necrosis [Figure 1a-e]. This indicates that PFDB have offered protection against TAM intoxication comparable to that of silymarin.

In summary, this study suggests that the oral administration of *D. bipinnata* significantly ameliorates TAM hepatotoxicity in rats. The polyphenolic fraction may be protecting the liver by free radical scavenging activity and thus preventing peroxidation of lipids of the endoplasmic reticulum. Moreover, this may be due to the presence of flavonoids in the fraction (whose presence was confirmed by HPTLC fingerprinting). Purification and characterization of the flavonoids present in the polyphenolic fraction are under progress. The study also rules out the presence of any antagonistic effect of the combination indicating a synergistic effect only.

References

1. Mayer SA, Kulkarni AP. Hepatotoxicity. In: Hodgson E, Smart RC, editors. Introduction to Toxicology. 3rd ed. New York: John Wiley and Sons Inc.; 2001. p. 487-90.
2. Jordan VC. Tamoxifen: A most unlikely pioneering medicine. Nat Rev Drug Discov 2003;2:205-13.
3. McCay PB, Lai EK, Poyer JL, DuBose CM, Janzen EG. Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals in vivo and in vitro. J Biol Chem 1984;259:2135-43.
4. Letférén P, Labbé G, Degott C, Berson A, Fromenty B, Delarue M, et al. Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice. Evidence that silymarin acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochem Pharmacol 1990;39:2027-34.
5. Akanitapichat P, Phraibung K, Nuchklang K, Prompplakul S. Antioxidant and hepatoprotective activities of five eggplant varieties. Food Chem Toxicol 2010;48:3017-21.
6. Joshi SG. In: Medicinal Plants. New Delhi, India: Oxford and IBH Co. Pvt. Ltd.; 2003. p. 318.
7. Amani SA, Nawal HM, Dereg KM, Solimon GA. Antiulcerogenic activity of extract and some isolated flavonoids from Desmostachya bipinnata. Nat Prod Res 2008;2:76-82.
8. Mohamed AR, Safwat NA. Antihelicobacter activity of a flavonoid compound isolated from Desmostachya bipinnata. Aust J Basic Appl Sci 2009;3:2270-7.
9. Medha MH, Lakshman K, Girija K, Ashok Kumar BS, Lakshmiprasanna V. Assessment of antidiarheal activity of Desmostachya bipinnata L. (Poaceae) root extracts. Biol Latinoam Caribe Plant Med Aromat 2010;9:312-18.
10. Panda S, Chaudhary NS, Patro VJ, Pradhan DK, Jana AK. Analgesic, antiinflammatory and antioxidant activities of Silybum marianum Coss (Asteraceae) in albino rats. Drug Invention Today 2009;1:150-3.
11. Sathiyam S, Bagul Y, Gupta S, Kaur H, Redkar R. Antioxidant effects of aqueous leaf extract and crude isolates of Murayna koenigii against in vitro ethanol-induced hepatotoxicity model. Exp Toxicol Pathol 2011;63:587-91.
12. Raj VP, Chandrasekhar KV, Vijayan P, Dhanaraj SA, Rao MC, Venkata JR, et al. In vitro and in vivo hepatoprotective effects of the total alkaloid fraction of Hygrophiula auriculata leaves. Indian J Pharmacol 2010;42:99-104.
13. Caballero F, Gerez E, Oliveri L, Falcoff N, Batlle A, Vazquez E. On the promoting action of tamoxifen in a model of hepatocarcinogenesis induced by p-dimethylaminobenzene in CF1 mice. Int J Biochem Cell Biol 2001;33:681-90.
14. Ahotupa M, Hirsimäki P, Pärssinen R, Mäntylä T. Alterations of drug metabolizing and antioxidant enzyme activities during tamoxifen-induced hepatocarcinogenesis in the rat. Carcinogenesis 1994;15:863-8.
15. El-Beshbishy H. The effect of dimethyl dimethoxy biphenyl dicarboxylate (DBD) against tamoxifen-induced liver injury in rats: DBD use is curative or protective. J Biochem Mol Biol 2005;38:300-6.
16. Kärki A, Mäntylä T, Hirsimäki P, Karlsson S, Toikkanen S, Hirsimäki P. Comparison of the effects of tamoxifen and toremifene on rat hepatocarcinogenesis. Arch Toxicol 2000;74:249-56.
17. Desai P, Nallani S, Sane R. Induction of cytochrome P450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. Drug Metab Dispos 2002;30:608-12.
18. Hellmann-Blumberg U, Taras TL, Wuzu GT, DepGregorio MW. Genotoxic effects of the novel mixed antiestrogen FC-1271a in comparison to tamoxifen and toremifene. Breast Cancer Res Treat 2000;60:63-70.
19. Recknagel RO, Glende EA Jr, Britton RS. Free radical damage and lipid peroxidation. In: Meeks RG, editor. Hepatotoxicology. Boca Raton, FL: CRC Press; 1991. p. 401-36.
20. González E, Roselló-Catalau J, Jawerbaum A, Sinner D, Pustowcr C, Vela J, et al. Pancreatic nitric oxide and oxygen free radicals in the early stages of streptozotocin-induced diabetes mellitus in the rat. Braz J Med Res 2003;33:1335-42.
21. Ostrowska J, Luszcz W, Kasacka I, Różanski A, Skrzylidewska E. Green tea protects against ethanol-induced lipid peroxidation in rat organs. Alcohol 2004;32:25-32.
22. Wohaieb SA, Godin DV. Starvation-related alterations in free radical tissue defense mechanisms in rats. Diabetes 1987;36:169-73.
23. Godin DV, Wohaieb SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 1998;184:223-31.
24. Kakkur R, Mantha S, Radhi J, Prasad K, Karla J. Antioxidant defence system in diabetic kidney. Life Sci 1997;60:667-79.
25. Seven A, Guelz S, Seymen O, Civelek S, Bolayrit M, Uncu M, et al. Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma. Yonsei Med J 2004;45:703-10.
26. Mak DH, Ip SP, Li PC, Poon MK, Ko KM. Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. Mol Cell Biochem 1996;162:153-8.
27. Lu SC. Regulation of hepatic glutathione synthesis: Current concepts and controversies. FASEB J 1999;13:1169-83.

Cite this article as: Rahate KP, Rajasekaran A. Hepatoprotective effect by active fractions from Desmostachya bipinnata statif. (L.) against tamoxifen-induced hepatotoxicity. Indian J Pharmacol 2015;47:311-5.

Source of Support: Nil. Conflict of Interest: No.