Pathological evaluation of *Polystichum squarrosum* (D. Don) fern in laboratory rats

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*Polystichum squarrosum* fern fed (30% w/w) rats showed moderate mortality, decrease in body weight, less body fat and splenomegaly. On post-mortem examination, significant gross lesions were not seen in sacrificed animals. Histopathologically, *Polystichum* fed rats showed dilated Virchow Robin’s space in brain, mild to moderate vascular changes like oedema, engorgement of blood vessels and haemorrhages in most of the visceral organs, interstitial pneumonia in lungs, focal necrosis and generalised vacuolative degenerative changes in liver, more haemosiderin deposition and presence of higher number of megakaryocytes in spleen, shrunken glomeruli, more peri-glomerular space and more number of glomeruli per microscopic field in kidneys, focal hyperplasia of urinary bladder and moderate to marked depletion of germinal epithelium and spermatids in seminiferous tubules of testes. Pathologically, progressive changes were observed only in liver, urinary bladder and testes on 180 days post feeding (DPF). One fern fed rat sacrificed on 135 DPF showed hepatic tumour which was diagnosed as hepatocellular carcinoma. The results showed that *P. squarrosum* produced almost comparable pathological changes/preneoplastic lesions as reported in bracken fern fed animals. Long term exposure studies (i.e. 2 yrs) are desired.

*Mature* *Polystichum squarrosum* fern is a fairly common fern in Kumaon hills and elsewhere. Under compelling situations or as fodder contamination it may be consumed by animals. Clinico-pathological effects of this fern were reported in rats and laboratory rabbits. In this study sequential pathological effects of *Polystichum squarrosum* in rats have been evaluated.

**Materials and Methods**

Mature *Polystichum squarrosum* fern with spores was collected from Mukteswar area, Ramgarh block, Distt. Nainital during September-October, 1999. The fern collected was shade dried and grounded in feed processing plant of the Institute. Fine grinding of fern was done in laboratory with the help of home mixy. Fern and its powder was stored in gunny bags at room temperature. Albino rats (60, 2-3 months old) weighing 100 to 150 g were selected and randomly divided into two groups of similar pre feeding body weight viz., *Polystichum* fed (37 rats) and control groups (23 rats). *Polystichum* fed group was provided with concentrate ration containing 30% ground fern for 180 days. All rats were served concentrate ration *ad libitum* twice a day. Six experimental and four control rats were sacrificed on 45, 90, 135 and 180 days post feeding (DPF).

Dead / sacrificed rats were weighed prior to necropsy examination. Brain and visceral organs of dead / sacrificed rats were systematically examined. Weight of liver, spleen and kidneys and visible pathological lesions were recorded. After thorough examination, representative tissue pieces from different organs viz. brain, heart, lungs, liver, spleen, stomach, intestines, kidneys, adrenals and testes were collected and preserved in 10 per cent formalin for histopathological examination as per Culling. Duplicate sections were stained by Perl’s method for determination of iron.

**Results and Discussion**

Out of 37 fern fed rats 8 (21.62%) died, while out of 23 control rats 3 (13.04%) died. Fern fed rats died on 7, 20, 32, 34, 83, 85, 111 and 121 DPF while control rats died on 16, 19 and 127 DPF. Comparatively higher mortality in *Polystichum* fern fed group indicated that it was toxic to the animals at 30% level. Somvanshi and Gounalan also reported that all rabbits fed with *Polystichum squarrosum* at 30% level died between 7 and 16 weeks.

*Polystichum* fern fed rats showed decrease in body weight throughout the experimental period (up to 180 DPF) (Table 1) which is in accordance with earlier reports. Loss in weight observed in fern exposed group was not due to less intake or inadequate protein intake. Rats of both groups were served *ad libitum* feed. Proximate analysis revealed that *P. squarrosum* fern contained 8.5 % nitrogen. On 90 DPF both groups of animals lost weight during severe winter
months of December/January. Weak and lowered weight animals from both fern fed and control groups were selected for sacrifice on this interval with an objective that remaining animals of comparatively higher weight will survive up to 180 DPF. Therefore weight loss in fern fed rats was due to toxic effects. Similar observations were available in bracken fern fed rats and guinea pigs.9

The relative liver weight per cent was significantly increased in Polystichum fern fed rats (3.70%) on 135 DPF as compared to controls (3.12%) (Table 1). But in other intervals, slightly lower liver weight was observed. Somvanshi et al.6 reported significantly increased liver weight on 50 DPF followed by reduction on 100 DPF in Polystichum fed rabbits. But contrary to this, decreased liver weight was observed in 7-16 weeks post feeding of Polystichum in rabbits.4 A great variety of plant can cause hepatic injury.10 Hepatopathy is suggestive of enhanced haemolysis and destruction of erythrocytes due to toxic effects of Polystichum fern.

Polystichum fern fed rats showed significantly increased spleen weight throughout the experimental period (Table 1). Similar results were reported in rabbits4,6. Splenomegaly may be due to lymphoid hyperplasia and accumulation of haemosiderin either free or loaded in macrophages as evinced on histopathological examination. This is also suggestive of toxic effects of Polystichum fern.

Histopathologically, Polystichum fern fed rats showed dilated Virchow Robin's space in brain, peribronchial and perivascular infiltration by mononuclear cells, focal hepatic mononuclear cells infiltration, hypersecretory activity in intestinal mucosa, degenerative changes and depletion of spermatogonial cells and spermatids in seminiferous tubules. These findings are similar to the observations of earlier studies in Polystichum fed rabbits4 and other species of ferns; Pteridium aquilinum in rats and Dryopteris juxtaposita in rabbits6,11. These vascular and degenerative changes were due to effects of toxin(s) present in Polystichum fern.

Changes in brain showed dilated Virchow Robin's space and prominent haemorrhages in cerebrum (Fig. 1). Vascular changes (Fig. 2), perivascular and peribronchial lymphoid infiltration / cuffing, alveolar thickening and interstitial pneumonic changes were observed in lungs on 45, 90, 135 and 180 DPF. These changes were prominent in later intervals (135 and 180 DPF). In conventionally reared rats, peribronchial lymphoid tissue is not uncommon feature. Such changes were also reported in murine mycoplasmal2. Interstitial pneumonia is generally caused by viruses. Possibly due to impaired immune status in fern fed rats, these changes were precipitated. Catarrhal bronchopneumonia were diagnosed in 2 fern fed rats on 180 DPF. One control rat also showed similar changes. The cause of these changes was not investigated and considered as incidental finding.

Table 1—Effects of Polystichum squarrosum fern feeding on body weight and organ biometry in rats
[Values are mean±SE from 6 rats in experimental and 4 rats in control group]
There was no appreciable progressive differences in histopathological alterations on 45, 90, 135 and 180 DPF intervals except in liver, spleen, urinary bladder and testes. Liver showed mild degenerative changes in earlier intervals (45 and 90 DPF) but on 135 and 180 DPF, marked generalised vacuolative changes were observed (Fig. 3). This may be due to cumulative effect of Polystichum toxin(s).

One fern fed rat sacrificed on 135 DPF showed hepatocellular carcinoma (Fig. 4). Although, it is difficult to say whether it was a spontaneous hepatic tumour or induced by fern feeding. Hyperplastic nodules in liver of rats treated with bracken fern was reported earlier. Prejean et al. reported that spontaneous hepatic tumours were rarely encountered in Sprague-Dawley rats and Swiss mice. Further that hyperplastic nodules of liver were found only in one out 264 rats in a study on spontaneous tumours.

Spleen showed marked haemosiderosis (Fig. 5) and hyperplasia of lymphoid follicles in the white pulp on 135 and 180 DPF. Perl's stained sections confirmed significantly large quantity of haemosiderin in spleen of fern fed rats (Fig. 6) than that of controls. On 180 DPF, spleen showed presence of more number of megakaryocytes (Fig. 5) than controls which is indicative of extra-medullary haematopoiesis in...
Spleen which further indicate suppressed bone marrow activity. Although these are general pathoanatomical alterations but they appeared due to toxic effects of *Polystichum* fern. Similar findings were observed in bracken fern fed rats and rabbits. On 180 DPF, shrunken glomeruli with more space between Bowman's capsule and glomeruli and more number of glomeruli per field, suggesting kidney atrophy were observed. Higher serum urea levels in *Polystichum* fern fed calves on 180 DPF were suggestive of kidney impairment.

On 135 and 180 DPF, transitional epithelium in the urinary bladder showed marked changes like vacuolation, hyperplasia (Fig. 7), hyper nuclearity and hyper cellularity. These alterations may be suspected as pre-neoplastic changes. Ushijima et al. suggested that toxic factors of the fern were eliminated through the kidneys as inactive form and got reactivated in the urinary bladder. This may be the reason for hyperplastic changes observed in the urinary bladder of some rats in the present study. Bringuier *et al.* reported higher incidence of bladder carcinoma in guinea pigs fed with diet containing 30% dried bracken fern for 150 days. Aswani Kumar reported nodular \ papillary hyperplasia of ureter and urinary bladder in 20 and 40% guinea pigs fed with bracken and cheilanthes ferns, respectively.

Progressive changes like depletion of germinal epithelium, spermatogonial cells and reduced population of spermatids was observed in testes of...
Polystichum fed rats (Fig. 8). This is correlated with findings of earlier study in bracken fed rats\textsuperscript{23,24}. Pathological changes in testes were suggestive of reproductive abnormality, which warrants further investigation. However, rabbits fed with different ferns were found to breed without any apparent abnormality (Somvanshi, 1999 unpublished data).

Rats fed with \textit{Polystichum} failed to develop neoplastic lesions either in ileum or urinary bladder corroborating earlier finding\textsuperscript{21-25}. Possibly it could be due to shorter period of experiment in the present study and loss of toxins in the fern during shade drying and processing of fern. However, during the present experiment, one rat showed liver tumour on 135 DPF and in urinary bladder of rats revealed hyperplasia / nodular hyperplastic changes which may be graded as pre neoplastic lesions. The results suggest that \textit{Polystichum squarrosum} produced almost comparable changes and preneoplastic lesions (rarely) those reported in bracken fern fed laboratory rats and guinea pigs. Long term exposure (i.e. up to 2 years) of \textit{Polystichum} fern studies will provide more information in relation with carcinogenesis of above fern.

References