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## DERMATOLOGICAL EFFECT AND TOXICOLOGICAL EVALUATION OF HUMIC ACIDS FOR TROPICAL USE IN SKIN WOUND HEALING AND SKIN DISEASES

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Humic acids are produced for the first time from low rank coals of Pakistan. Humic acids and its salts are used as therapeutic agents especially in veterinary medicine. Humic acids is a very economical product due to its production from indigenous raw material. The evaluation of humic acids in dermatological applications for skin affection is carried out to find about its feasibility of utilization in pharmaceuticals related to dermatology.

**Key words:** Lignites, Humic acids, Dermatology.

### Introduction

Humic acids is a widely distributed natural product found in soil, peat and lignitic coal and is used as a therapeutic agent, especially in veterinary medicine [1-3]. Humic acids is formed in lignitic coal through a slow progressive oxidation of the plant residue, followed by physiochemical and geochemical alteration [4].

Humic acids is alkali soluble polymeric organic acids with aromatic structure, substituted by carboxylic, phenolic, amino, hydroxyl and alkyl groups, linked together through ether linkages [5].

Humic acids is derived from lignitic coal and has approximately the some pharmacologically active structural compounds as volatile coal products i.e. coal tar and tar acids which are widely known as dermicidal agents and are used in various skins affections like eczema, psoriasis, dandruff etc. in the form of lotion, paste and ointments [6]. The activity of coal tar and tar acid ointments is attributed to the phenols and hydroxy acids, phenols are known to have a dermicidal action.

Humic acids is a coal derived product with acidic and phenolic groupings only with added advantages. It is not a volatile coal product, it contains sulphur in its structure and sulphur is a known antifungal, antibacterial agent and is widely used in dermatological applications [7,8]. Due to its large surface area and grouping humic acids possess marked adsorbing and buffering powers [9], thus making it a very valuable dermatological agent for skin wound healing. Due to this adsorbing power, humic acids and its salts are used for detoxication for heavy metal poisoning [10]. It is shown to adsorb large amounts of heavy metals in the gastrointestinal tract [11]. It is used as an antacid and has a protective action against gastric lesions induced in rats [12]. It is used as an anticaries agent in dentistry [13]. Humic acids and its derivative are used in rheumatic diseases and degenerative diseases

of the spine [14]. As a phenolic polymer it is known to inhibit herpes virus [15]. Sodium humate is reported to be used for the skin wound healing [16] and chronic gastric ulcer in rats [17,18]. The humic acids due to their dermatological action are also used in skin cosmetics [19,20].

The chemical composition and ultimate analysis of humic acids vary with the nature of different coals. Pakistan's lignitic coal contain high percentage of sulphur (5-6%). The humic acids produced from lignitic Pakistan coal contain a higher percentage of sulphur and should have a higher antifungal/antibacterial activity compared to humic acids derived from low sulphur coals.

The literature survey indicated that the most common therapeutic effects of humic acids are due to its buffering, adsorbing, bactericidal, antiviral, antifungal and antiastringent properties, these properties are essential for effective dermatological treatments. So far no work has been carried out on humic acids produced from lignitic Pakistan coals. It is an objective of the present paper to evaluate, for the first time, the therapeutic efficiency of high sulphur humic acids and its salts produced from the lignitic coal of Pakistan for skin diseases and skin wound healing.

### Experimental

**Preparation of humic acids.** Humic acids was prepared through the mild oxidation of lignitic coal and purified by a standard method. The elemental analysis was obtained using a Leco Model CHN-600 elemental analyzer. Sulphur was determined by a Leco Sulphur Determinator Model SC-132. Ash was determined by Leco Mac-400. FT-IR spectra was recorded on a Perkin Elmer FT-IR Spectrometer Model 1800 using KBr. Elemental analysis of humic acids was as follows:

C	H	N	O	S	Ash
57.30	4.43	6.86	30.17	1.24	6.13



The elemental analysis of humic acids showed 1.24% sulphur in its structure. The phenolic groupings were confirmed by 3400  $\text{cm}^{-1}$ , 2920  $\text{cm}^{-1}$  and 2860  $\text{cm}^{-1}$  bands and carboxylic grouping at 1720  $\text{cm}^{-1}$  in the FT-IR spectra.

**Preparation of humic acids cream.** A cream was prepared by mixing humic acids (0.5 g) suspension in distilled water and petroleum jelly (200 g), the latter is usually used as a base in the formulation of ointments and contains other substances of therapeutic value. It enables medicaments to penetrate into the skin. The contents were mixed into fine cream in a pestal mortar and stored for application.

**Animal experimentation.** The acute dermal test was performed on sixteen adult healthy albino rats using the criteria of Loomis [21]. The area over the back of each animal extending

surgeon Mr. M.H. Pirzada\*. Animals were divided into three groups, each group was comprised of 4 animals and housed in separate cages. The efficacy of humic acids test cream was compared with the standard furacin ointment. (Smith and Klien, active constituent nitro pirazone 0.25%) widely used for skin wound healing and skin infections and the control petroleum jelly. Before the application of the test and the standard cream infected portions were washed with luke warm water to remove dust and other adhering material. The cream applications were made depending upon the size of the wound thrice daily for fifteen days and observations were made daily as shown in Table 1. Photographs of all the animals were made before and after treatment of the skin infection as shown in Figs. 1-3.

TABLE 1. SHOWING COMPARATIVE STUDY OF THE HUMIC ACIDS AND FURACIN CREAM ON THE VARIOUS STAGES OF WOUND HEALING.

The drying of wound (days)		Appearance of the granular tissues (days)		Size of the wound decreasing (days)		The degree of erythema decrease		Complete wound healing (days)	
Humic acids	Furacine	Humic acids	Furacine	Humic acids	Furacine	Humic acids	Furacine	Humic acids	Furacine
5	7	6	8	7	10	8	12	10	15

from the base of the neck to the hind quarters was shaved. The shaved animals were then divided into two equal groups. To one group an area of approximately 2 square inches of the bare skin was abraded by making minor incisions through the surface layer of cells without producing bleeding. Test cream was applied (0.5 g approx.) over the shaved area of the skin of both the abraded and non-abraded animals. Second group was kept as control and only petroleum jelly was applied. The entire trunk of each animal was wrapped in a non-absorbent binder for the subsequent 24 hrs. After 24 hrs, the binder was removed and the area of exposure was evaluated. The treatment was repeated after 24, 48 and 72 hrs.

The criteria used to diagnose toxicity was by noting the inflammatory response effects of humic acids cream on the skin i.e. the degree of erythema and oedema on the site of application of the cream. The scoring was performed on each rabbit for the degree of erythema and the degree of oedema to get the primary irritation score using the criteria of Loomis [21] the results were compared with control. In all the rabbits no erythema or oedema was observed i.e. the primary irritation score was zero showing that the humic acids cream was non-toxic.

After performing the acute dermal test the cream was tested on wounded and skin infected rabbits. Most of the animals were suffering from fungal/bacterial infections and myxomatosis as diagnosed by the veterinary physician and

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### Results and Discussion

The dermatological studies on animals indicated that the humic acids test cream was more effective than the standard marketed cream furacin (Smith and Klien). As shown in Table 1 the initial and complete cure duration of humic acids cream was 5 and 10 days respectively and while in furacin ointment the initial and complete cure duration was 7 and 14 days respectively. While petroleum jelly only removed roughness of the diseased/wounded skin. In the end of the study all the experimental animals of humic acids and furacin cream treated animals were cured.

Humic acids as reported in the literature and shown by elemental and FT-IR spectra is a phenolic organic polymer with sulphur and carboxylic groupings in its structure. Sulphur is a known bacteriocidal and antifungal agent. It is chiefly used in the treatment of various parasitic skin diseases and carries a strong dermatological action. Phenolic groupings also carry bacteriocidal action. Pure sulphur when directly applied to skin has no effect and phenols are strongly irritating to the skin. In humic acids both sulphur and phenolic groupings are present in such a form that make humic acids a strong dermatological agent possessing bacteriocidal and antifungal properties with no irritating effects as shown by experiments. It is produced through a single step from lignitic low rank weathered coal of Pakistan which is least suitable to be utilized as fuel. The raw material is not only indigenous but is also very cheap for its production. It is used in low 0.25%



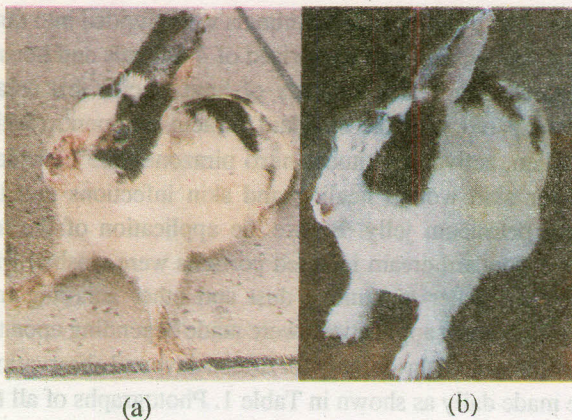


Fig. 1. Infected rabbits: (a) Before treatment; (b). After treatment with Humic acid cream.



Fig. 2. Infected rabbits: (a). Before treatment; (b). After treatment with Furacin ointment.

concentration i.e. 0.5 g per 200 g of petroleum jelly making it more economical. Pakistan is an agricultural developing country, the raw material for pharmaceutical products is imported and thus medication is expensive. Animals in the farms, poultry houses and bred for slaughter can be successfully and economically treated with humic acids cream for curing skin affections in animals, which otherwise in many cases are left untreated due to the high cost of medication.

#### Conclusion

Humic acid is an effective/economical dermatological agent produced from indigenous coal and may be utilized in veterinary medicine for curing skin diseases and skin wound healing.

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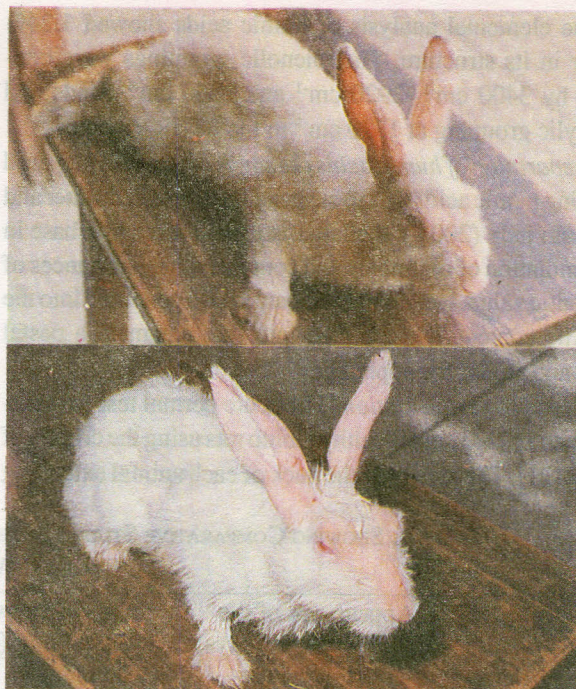


Fig. 3. Infected rabbits: (a). Before treatment; (b). After treatment with Petroleum jelly.

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Two genotypes of spring and winter wheat were studied for their potential use in tissue culture. Calli were initiated from mature embryos of Linamar and Skog's (L2) basal medium containing 4.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 2% sucrose and 1% agar. Cultures were maintained on the same L2 medium with 2,4-D reduced to 0.2 mg/L by reducing 2,4-D to 0.1 mg/L and adding 0.1 mg/L indole-3-acetic acid (IAA) and benzylaminopurine (BAP) respectively. Complete plants were regenerated by transferring the calli to 2,4-D-free medium. Significant genotypic variation was observed for callus induction frequency, callus formation and the potential for plant regeneration. Cultures of ten genotypes remained morphogenic for 210 to 270 days. Three genotypes gave 3 callus lines after 425 days. For the first time wheat genotypes have been identified which were able to regenerate plants after 900 days in culture (PI-47802, PI-39938, PI-47801). Of the 20 genotypes, PI-47802 and PI-47801 yielded the highest number of callus lines (PI-47802 and PI-47801 gave the highest number of regenerants). A total of 297 plants were regenerated and seeds were obtained. The genotypic effect on callus induction, long-term maintenance and plant regeneration in wheat genotypes is discussed.

**Key words:** callus, tissue culture, long-term, regeneration, maintenance

#### Introduction

The technology of growing cereal plants from either somatic or haploid cells has provided exciting new potential for plant improvement. Currently, all major cereal crops including rice (*Oryza sativa*) [1,2], barley (*Hordeum vulgare* L.) [3], maize (*Zea mays* L.) [4], pearl millet (*Pennisetum americanum*) [5], sorghum [*Sorghum bicolor*, *Sorghum and wheat* (*Triticum aestivum* L.) [7-9] have been grown in cell culture programmes with various degrees of success.

Several reports are available in the literature on callus induction and plantlet regeneration from various tissues of different wheat genotypes [7,9-18] but much less information is available on their potential for genoplasm improvement.

The search for specific genotypes that are capable of morphogenic callus production, high rates of plant regeneration and long-term maintenance is an important step towards the application of tissue culture techniques to agriculture. The objectives of this study were to determine the response of wheat genoplasm to *in vitro* culture, to develop techniques to regenerate plants from callus cultures and to establish long-term regenerable cultures. The genotypic effects on callus induction, growth response, regeneration and long-term maintenance and regeneration potential of wheat genoplasm are discussed.

#### Materials and Methods

Callus induction. Seeds of 20 wheat genotypes (Table I) were obtained from the Small Grain Storage Lab, Beltsville, Maryland, USA.

Embryos of 20 wheat genotypes were surface sterilized for 30 sec. in 70% ethanol, then washed vigorously (using magnetic stir bar) with a 20% chlorox (commercial bleach at 2.5% sodium hypochlorite) solution plus a drop of Tween-20 for 30 min followed by rinsing for 3 min in 0.05 mercuric chloride solution. After six rinses, seeds were left to soak for 8-12 hrs in the seventh rinse of sterile distilled water. Mature embryos were dissected out and were plated in vials containing basal salts of Linamar and Skog's (L2) [17], 4 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 2% sucrose solidified with 1% agar. The medium was adjusted to pH 5.5 and autoclaved for 15 min at 12 psi and 120°. After 30 days in culture the 2,4-D level was reduced to 0.2 mg/L. The cultures were transferred to fresh medium at 30 day intervals thereafter. Embryos which germinated were discarded. On an average, 100 embryos were used per genotype.

Cultures were maintained in an environmentally controlled room at 22 ± 2° in continuous light of 2000 lux at a height provided by four General Electric wide-spectrum fluorescent bulbs.

Callus induction frequency for each genotype was recorded after 30 days in culture. For callus growth measurement, calli from 20 jars were weighed individually at each passage.

Plant regeneration. The calli were divided into approximately 2 mm pieces and placed on L2 medium with 2,4-D levels reduced to 0.1 mg/L. The 2,4-D level promotes shoot development but partially inhibits root development. Calli that produced shoots were then transferred to large jars (40 mm in